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DAVID A. RYTAND, *Editor*
Stanford University School of Medicine

WILLIAM P. CREGER, *Associate Editor*
Stanford University School of Medicine

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PREFACE

One may note a trend in this and recent volumes of the *Annual Review of Medicine*, toward more concentrated discussions of relatively limited fields and also that writers from other lands are contributing to the desired broad coverage of medical science. To all of those experts whose reviews comprise this volume, we are deeply grateful, and we give thanks as well to Miss Beryl V. Daniel, Editorial Assistant.

R.E.C.	G.J.D.
W.P.C.	M.A.H.
W.C.C.	D.A.R.

E.A.S., Jr.

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VIRUS DISEASE: SOME ASPECTS OF HOST AND TISSUE SPECIFICITY[1]

By F. B. Bang

Departments of Pathobiology and Medicine, Johns Hopkins Medical Institutions, Baltimore, Maryland

Virus infections were surely a part of man's experience long before the species was separated from the other anthropoids. As human communities increased in size greater opportunities for survival were given to these small cellular parasites—and greater opportunities of transfer from animal reservoirs into the human population. It is entirely possible that new types of human virus infections are constantly being produced. The tremendous increase in virus research which has occurred in the last ten years has, however, brought forward a mass of data that is only beginning to be digested. The publication of the third edition of Rivers' compendium of *"Virus and Rickettsial Diseases of Man"* (1), the emergence of a new three-volume text on viruses (2), the appearance of a new journal from the Communist countries, *"Acta Virologica,"* (3) published both in Russian and English, the continued publication of *"Advances in Virus Research"* (4), and of *"Progress in Medical Virology"* (5) not only reflect this vigorous adolescence but make a considered review difficult.

While most of the latter were being compiled (though not published) a pandemic of influenza circled the world, and nearly every virologist who had chick embryos or tissue culture tubes handy has written about it Several preliminary reviews (6, 7) have been published but there has not been time to assess the pandemic as a whole

In the hope, then, that this review might be other than a modernized bibliography we have purposely excluded large portions of the recent literature on such virus diseases as, for example, poliomyelitis, Coxsackie, and adenoviruses. This omission has been made easier by the availability of the above-mentioned texts as well as the authoritative review of "Newly Recognized Respiratory Tract Viruses," by Huebner, Rowe & Chanock (8). Our emphasis then will be on (*a*) the pathogenesis of certain respiratory tract infections; (*b*) the pathogenesis and the production of symptomatology of central nervous system virus infections; (*c*) the general problem posed by chronic virus infections; and (*d*) animal-human virus relationships. A scattering of miscellaneous contributions will also be included, with no attempt to force them into the framework

Pathogenesis of Respiratory Tract Virus Infections

Infectious agents inoculated by a more or less direct implantation of large particles on the upper respiratory tract may produce localized infection.

[1] The survey of the literature pertaining to this review was concluded in August, 1959.

The means by which the mucus-ciliary complex responds to this historic insult is poorly understood. Experimentation on man continues to be important (9, 10) in demonstrating the etiological significance of recent candidates for the title of the "cause of the common cold" Tyrrell & Bynoe (11), from the Common Cold Research Unit at Salisbury, have reported that the J.H. virus isolated by Price infected 11 of 55 volunteers who were inoculated with it, that the virus multiplied in the nose and throat, and that there was little relation between antibody titer and experimental susceptibility. Price *et al.* (12) reaffirm the role of the J.H. and 2060 [Pelon *et al.* (13)] viruses in producing mild upper respiratory disease in a large proportion of the population, but found evidence of their presence in only 12 per cent of 901 upper respiratory infections at the time of study. Jackson & Dowling (14) found that of 1034 subjects inoculated with common cold washings, 42 per cent developed colds as compared with 10 per cent of those receiving buffered solutions, a figure very close to that of the British workers (9) in Andrewes' group

However, this gives us a little knowledge of the actual pathogenesis For this reason some animal experimentation has been carried out on the factors involved in natural upper respiratory tract infection of animals (15) and recently a large text by an otolaryngologist (16) and a review of the comparative anatomy of the region (17) have been published.

Ciliated and mucous cells are discarded into the overlaying mucous current as they are damaged or destroyed in the course of growth, differentiation, and repair. Thus, as large numbers of them are infected and damaged, they are sloughed off and may be found in the nasal secretions of upper respiratory infections or in the sputum of acute generalized respiratory virus infections. This latter point was well documented by Hers & Mulder (18) several years ago, and has been used by several Russian virologists in the differential diagnosis of influenza by staining the exudate and searching for eosinophilic cytoplasmic inclusions (19) It has even been suggested that these inclusions represent a cellular defense mechanism (20). The occurrence of these degenerate ciliated cells in the respiratory mucus has been given the name "cilio cytophthoria" which seems hardly justified until some degree of specificity of lesion besides their association with viral disease is established (21) The staining of the ciliated and mucous cells with specific fluorescent antibody as was done by Liu (22) seems of more likely general diagnostic value.

Destruction of human ciliated epithelial cells by influenza and the sloughing of these cells into the overlying mucus has been demonstrated in organ cultures of human embryonic trachea (23). Presumably, this reflects the course of a mild, possibly asymptomatic, influenza infection.

The emergence of pandemic, but relatively mild, "Asian" influenza re-emphasized several old problems in the pathogenesis of influenza. These may be separated into the (*a*) pneumonia and the possible pneumotropism

of the virus; (*b*) the bacterial complications; and (*c*) the neurological complications and their genesis.

Pneumonia.—Pneumonia, which is apparently caused by the virus alone, has been reported in a number of series (24 to 27). The evidence for this is both pathological and clinical. The absence of bacteria and the recovery of virus from the lung in relatively large amounts, when combined with necrosis of alveolar epithelial cells, as described by Hers, seems clear enough. The reason for the pneumonia is less easily determined. A natural virus infection of animals may be either limited to the upper respiratory tract or spread through it by aerosolation. It is not known whether any aerosol spread of pandemic influenza took place, and tests with the virus itself on animals have no bearing on the human infection Many of the series of case reports point to the effect of cardiac disease (28), pregnancy, and chronic respiratory disease as predisposing causes of influenzal pneumonia, and it seems clear that people with mitral stenosis, etc, were particularly susceptible. Such susceptibility might well be explained by the excess pulmonary fluid which has been recognized as a factor in the spread of the virus (29). It has also been proposed that this influenza strain has a direct pneumotropic tendency, i e, it multiplies in and destroys the alveolar and bronchiolar epithelium of man more readily than do other strains. Demonstration of this as a characteristic of the virus would need more elaborate tissue culture or animal experimentation than has yet been forthcoming.

Bacterial complications—Possibly because of both increased interest in and greater prevalence of pathogenic staphylococci, this organism was found as the most frequent associated pathogen (24, 26, 27) in general, although pneumococci were also active (24). There has been no evidence of actual synergism between the influenza virus and the staphylococcus, and the relative importance of ciliary destruction (which has been clearly shown in the absence of bacteria) and of pulmonary edema, etc., in preparing a more favorable condition for the growth of the staphylococcus is unknown The association of staphylococcus and influenza A (not Asian influenza) was studied in monkeys by Verlinde & Makstenieks in 1954 (30). With the limited number of animals available, quantitation of the effect was difficult. The desquamation of the epithelium and the peribronchial edema and intra-alveolar edema were increased by the presence of coagulase-positive staphylococci. The combination produced a more extensive epithelial necrosis which was mostly limited to the bronchioles but which sometimes encroached further on the respiratory tree In the latter case, it was then accompanied by bronchopneumonia The authors conclude that though the two together produced more lesions, there was no evidence of synergism Thus, both in the naturally occurring and in the experimental disease, the association of the two in one host is dangerous, but there is no evidence of a synergism similar to the "complex infection" produced by swine influenza virus in association with *Hemophilus influenza suis*

Neurological complications.—Ingress into the central nervous system from the respiratory tract may come either by way of the olfactory bulb or by way of the blood stream. Two other members of the myxovirus group of viruses (mumps and Newcastle) are well recognized as pathogens of the central nervous system. One of the classical laboratory strains of influenza virus is a "neurotropic W.S." strain which may produce extensive viremia in new born mice (31). Recently, viremia has been reported with other strains (32). Viremia had not been reported in man with influenza until the recent pandemic. In laboratory isolation of myxoviruses by egg inoculation it is too well known that if passage of virus is required, contamination is almost inevitable if isolations are made in a room in which the same strain of virus is under study in embryos. Precautions against this are difficult. In the study reported by Kaji *et al.* (33) in which the virus of Asian influenza was recovered from (*a*) the tonsil; (*b*) spleen and lymph nodes; and (*c*) the liver, spleen, kidney, and heart of 3 of 15 fatal cases of influenza, precautions were taken to prevent contamination of the tissues at autopsy, and the virus was twice reisolated from the stored frozen tissue by the amniotic injection of embryos. The virus was also recovered from a 1-to-500 dilution of the original spleen. Although virus was not recovered from the blood, presumably only blood dissemination could produce the spread

A wide variety of neurological sequelae to influenza have been reported, ranging from transient neurological signs to coma and death. At autopsy there are few inflammatory changes, which agrees with the lack of cells in the spinal fluid. One striking case reported by McGill & Goodbody (34) showed at autopsy a generalized hemorrhagic encephalitis with the predominant lesion present in the white matter A careful study of the pathogenesis of the blood-borne encephalitis produced by the W S neurotropic strain of influenza in chick embryos shows lesions very similar to these (35).

The need for demonstrating that the various encephalitides which occur at the time of pandemic influenza are actually caused by influenza virus is great (36 to 40). The number of cases reported as occurring during or immediately after proven influenza epidemics seems to be more than chance coincidence The similarity of the experimental and natural lesions, the recognized neurotropisms of other members of the myxovirus group, and the laboratory selection of highly neurotropic strains of influenza A makes the circumstantial evidence strong The recovery of virus from the central nervous system has been reported (41) but more careful isolations are needed.

Pathogenesis of Central Nervous System Infections

Clinical virologists have in the past accepted the idea that most of the easily recognized viral infections of the brain or spinal cord could not be diagnosed in terms of specific etiology, and that about 10 per cent of cases of aseptic meningitis were attributable to a known virus. The great increase

in available agents combined with greatly improved routine diagnostic tests has offered a real challenge, and a contribution from the Veterans Hospitals and the Army Medical School showed that between the years 1953 and 1958, 71 per cent of the 430 cases of aseptic meningitis were identified and 72 per cent of the 139 cases of encephalitis (42). Cases of aseptic meningitis were caused principally by Coxsackie B, mumps, ECHO, poliomyelitis, lymphocytic choriomeningitis, and herpes simplex, in that order. The cases of encephalitis were chiefly attributed to mumps, lymphocytic choriomeningitis, and arthropod-borne viruses. Of particular interest was the rise in cases of Coxsackie and ECHO viruses during midsummer and early fall, whereas mumps had its greatest prevalence in the spring.

A clue to the question of "orphan viruses in search of a disease" was provided by Arnold & Enders' (43) demonstration that two ECHO viruses which have been associated with cases of acute paralysis produced, when injected intrathecally into monkeys, clinical signs of muscle weakness and pleocytosis of the spinal fluid along with antibody response

Arbor viruses—There is little need for a complete review of recent work on these arthropod-borne agents. The Rockefeller Foundation has, in the last few years, collected and studied these agents all over the world and they have been extensively reported by Theiler & Casals (44). Work (45) has reviewed the experience following the sudden outbreak of Kyasanur Forest Fever in India; and a complete review of Russian Spring-Summer encephalitis has been published by Smorodintsev (46).

The variety of exotic names which are attached to the different viruses reflect temporary confusion about the exact relationships of the different members Casals' work is rapidly bringing order into the group but, in the meantime as outbreaks occur and agents are isolated, new distant spots gain recognition An unusual outbreak of fever occurred in a group of Okinawan pioneers who were attempting to settle in the Bolivian rain forest (47). Of a group of some 400 almost half had "jungle fever," consisting of the sudden onset of fever, headache, profuse sweating, and polyuria. There were 15 deaths. A new virus was isolated from the blood of patients by Schmidt *et al* (48) and was shown to be the cause of some of the fevers. Yet the outbreak, which appeared in October of one year and lasted until July of the next when the colony was abandoned, was apparently of multiple etiology The virus called "Uruma" virus belongs to Casals' group A and is clearly related to Mayora virus.

The recognition of a number of cases of tick-borne meningo-encephalomyelitis (49) in Sweden serves as a geographic intermediate between the louping ill of Scotland and the Russian Spring-Summer encephalitis. In a study of 66 patients who had serological evidence of infection with this virus, 70 per cent reported tick bites and 75 per cent had a diphasic febrile course (50) In Russia, continued emphasis is also placed on goat's milk as a possible source Levkovich & Pogodina (51) report 102 cases of alimentary tract infections primarily in small families living in the city away

from the likelihood of tick bites, but known to be consumers of goat's milk.

Despite extensive studies on the encephalitides of the United States, particularly eastern and western equine encephalomyelitis, there is still considerable haze surrounding problems of winter reservoirs, transmission, etc. When the ordinary garter snake is infected by the mosquito vector, *Culex tarsalis,* there is a viremia in the snake for 4 to 20 days, with as much as 10^6 or 10^7 virus being recovered from the blood (52). Colorado tick fever virus, when transmitted to porcupines by infected ticks, produces a viremia which lasts from 25 to 50 days (53). Undoubtedly, as new viruses are discovered and new animals inoculated, more peculiar combinations will be found, each needing further epidemiological evaluation

Analysis of clinical symptomatology.—Despite the tremendous advances in neurophysiology, there have been relatively few attempts to apply either techniques or ideas to an analysis of the variety of peculiar symptoms produced by virus infections of the central nervous system. This, of course, cannot be done on man and, again, reasoning must be by analogy with experimental diseases of animals

A uniformly fatal and peculiarly horrible disease of cattle, appropriately called "mad itch" (pseudorabies), has been studied in laboratory animals for some years. The rhythmical progressive scratching localized to the site of inoculation has been explained as part of the progress of the virus up the sensory nerve to the dorsal root ganglia. When sympathetic ganglia are infected by appropriate inoculation, these ganglia may be studied *in vitro.* Dempsher, Larrabee *et al.* (54) showed that spontaneous discharges coming as rhythmical bursts of activity were a regular occurrence in such infected ganglia, and that they might be studied both in an *in vitro* bath or in the intact animal. In this first report the relation of the virus multiplication and pathology to the symptomatology was established, and the progression of the discharge first over the postganglionic fibers, then over the preganglionic fibers was followed The suggestion was made that the acetylcholine mechanism was involved since the activity seemed to be at the junction between nerve and cell (55). In further studies of this system (56), Dempsher *et al.* explored the possibility that the presynaptic nerve endings are superirritable, and that the diseased cells release acetylcholine because the virus infection is interfering with the normal action of an inhibiting system. Evidence for this idea was (*a*) blocking normal preganglionic transmission either by cocaine or by cutting, evoked spontaneous activity in those ganglia which were early in their infection and had not developed activity; (*b*) activity was increased if it was already minimal; (*c*) gamma aminobutyric acid, norepinephrine, and epinephrine caused initially a simultaneous and synchronous increase in activity of both pre- and postganglion nerves. Regardless of the eventual explanation of the phenomenon, this model of cell pathology is clearly of importance in the initial investigation of the rhythmical irritation characteristic of the disease.

Russian work on the relation of virus activity to neurophysiology has

simultaneously followed the same general interest. Medvedeff (57) apparently demonstrated in 1954 that the influenza virus exerts an action on the superior cervical ganglion of cats. This was done by perfusion of the blood vessels leading to the superior cervical ganglion and studying the response of the "third eyelid" Ado & Alekseyeva (58), from the same laboratory in Moscow, studied the action of influenza virus on the superior cervical ganglion of ferrets. The ganglia were taken from animals which were (*a*) healthy, (*b*) in the acute phase of infection (3 days), and (*c*) immune. Influenza virus was reported as depressing or inhibiting the action of acetylcholine on normal ganglia which, with ACh, gave off spontaneous discharges. The addition of influenza virus (allantoic fluid) to ganglia from infected ferrets yielded greatly increased spontaneous activity in the post-ganglionic fibers, and the virus increased the susceptibility to acetylcholine. Ganglia from immunized ferrets behaved much like those from the acutely infected ferrets. Various controls and comparisons were made with normal allantoic fluid, influenza B, and herpes virus Influenza virus apparently had no effect on the sensory sensitivity of skin receptors. If this work is substantiated it is clear that a heavy infection of the respiratory tract in the ferret (which is highly susceptible) leads to functional changes in at least one part of the sympathetic chain which drains the respiratory tract. The significance of this for man is unknown, but it must be remembered that the respiratory tract of the ferret presents a huge involuted surface which is severely affected by the experimental disease Thus, there is a much greater chance for infection of the ganglia in the ferret by drainage either by way of the blood stream or possibly through the sympathetic nervous system connections in the upper respiratory tract.

Many of the clinical abnormalities and symptom complexes produced by viruses, and which are difficult of analysis in man, may be seen either spontaneously or experimentally in animals Presumably, the most fruitful infections to study would be those of primates Bogaert (59) reported several types of spontaneous encephalitides, presumably viral, in primates in the Antwerp zoological garden. Symptoms included severe behavior disturbances, anxiety, general and Jacksonian epileptic seizures, various myoclonic movements, and postural tremors Autopsy showed severe encephalitis of the brain stem which spread anteriorly to the hypothalamus

Myoclonic (rhythmical, jerking, muscular) movements are characteristic of Newcastle disease in chickens, mice, cats, and monkeys. In a continued analysis of this activity it was found (60) that typical myoclonic epilepsy was a common part of this disease. A histologic analysis showed that the virus apparently spread along preferential nerve pathways, as had been shown previously for other viruses by other workers. There seemed to be a clear dependence of localization of both myoclonus and paresis on the route of inoculation. Electroencephalography showed no evidence of cerebral dysrhythmia and acute decerebration did not alter the character or rhythm of the myoclonus. The focal origin of these rhythmical efferent im-

pulses to the muscle groups was best demonstrated by further experimental surgical procedures (61). Some 13 cats infected through cranial routes were made acutely spinal by high cervical transection at the level of the allantoic-occipital junction In all but one the character, distribution, and rate of myoclonus in limbs and trunk were unaltered. Complex spinal reflexes were observed and these spinal animals were able, without artificial respiration, to maintain adequate pulmonary ventilation for hours solely by myoclonic contractions. Additional acute transections were performed in animals in which the myoclonus resulted from virus inoculation directly into the spinal cord. In six cats infected in the cervical region, the myoclonic movements developed initially in the forelimbs and later involved the hindquarters. Midthoracic cord transection then abolished the myoclonus completely in the lower extremities but had no effect on muscular contractions in the forequarters Conversely, in seven cats inoculated into the midthoracic or lumbar spinal cord, myoclonus made its first appearance in the hind legs and subsequently in the forelimbs. In these animals acute high thoracic transection abolished the movements in the forequarters, leaving those in the hind limbs unchanged.

These observations, combined with a recent study by Feldberg & Luttrell (62) which showed that intraventricular inoculation of the virus often produces myoclonus of the tongue and that this may be abolished by local injection of small amounts of paraldehyde or chlorpromazine, clearly demonstrate a focal origin from groups or nuclei of nerve cells.

Chronic Virus Infections

Continued advances in the study of viruses in cells and animals have made the clinical virologist aware of the great difficulties of assigning etiological significance to a particular agent when it is isolated from the patient. This is particularly true of chronic infections in which an antibody rise no longer occurs and therefore gives no circumstantial evidence of the presence of virus. On the one hand, virus may be repeatedly isolated from apparently healthy organs, as witness the adenovirus from the adenoid tissues obtained at operation; on the other hand, classical virus infections such as chicken pox or herpes zoster, or both, have yielded an agent which kills cells and spreads from cell to cell in tissue culture, but which has not been recovered from the tissue culture fluid or from homogenates of the infected cells Thus, virus may be recovered in the absence of disease, or infectious virus may be absent in the presence of specific viral lesions

In a series of young children, Rowe *et al.* (63) have shown that the virus of human salivary gland disease may be recovered repeatedly from mouth swabs over a period of two to five months and from 8 to 24 months after the infection originally produced antibody. The virus was also present in the urine as long as 15 to 24 months after neutralizing antibody had first been demonstrated. Furthermore, virus was found only in those children

who had complement-fixing antibody. The responsibility of this virus in disseminated disease is becoming clearer (64). Newborn infants may have jaundice, petechiae, and hepatosplenomegaly leading to death Older infants and children develop gastrointestinal, upper respiratory symptoms with a prolonged course, usually fatal. The disseminated form appears in adults in association with diseases of the hematopoietic system.

Bredsky & Rowe (65) subsequently showed that the mouse analogue of this virus can be recovered from the salivary gland one year after the original infection of the mouse, while typical inclusions were not found later than 60 days.

Hepatitis is perhaps the most serious of human chronic virus infections, and it is probable that little advance will be made until this agent can be cultivated in tissue culture or can produce disease in animals. It was suggested by Braunsteiner *et al.* (66, 67) that the agent had been seen in their electron microscopic sections of biopsy materials. The published pictures are not similar to known viruses, and may represent cellular changes unrelated to viral multiplication

A hemagglutination test developed by Havens (68), which is dependent upon the treatment of sera with acetone and the agglutination of chick cells, has a high apparent correlation with the acute stage of the infection, but it may also be positive in other situations. It is not suggested that the hemagglutinin is the virus particle.

The virus of canine hepatitis has no apparent relationship to human hepatitis (69). From a recent study of a familial infection with another virus (hepatoencephalomyelitis) this appears also to have no relationship to human hepatitis (70)

Human tumor viruses—The increasing knowledge of the variety of tumors produced in animals by viruses, and the growing numbers of such known agents, have intensified the probability that such an agent will be found in man. It is, of course, well recognized that benign lesions like warts are caused by viruses, and a recent electron microscopic study of those which occur in the larynx showed large numbers of fully developed viral particles in the cells from papillomas from two patients (71). These have some similarity to the particles seen in the warts studied by Bunting (72).

Evidence for viral etiology of human malignancies will then need continued careful consideration Present data may be considered under three headings. Electron microscopy has so far shown no or, at best, equivocal particles (73, 74). This, however, is not completely out of keeping with animal tumors, for the most malignant viral tumors may show few if any demonstrable viral particles if relatively little necrosis has occurred. However, a variety of animal tumor viruses have been seen in electron microscope sections of spontaneous tumors (75). There are two reports of the isolation of infectious virus from human tumors The first, (76), from cases of Hodgkin's disease, seems to be a real virus, but aside from the usual possibility

pulses to the muscle groups was best demonstrated *by further experimental* surgical procedures (61). Some 13 cats infected through cranial routes were made acutely spinal by high cervical transection at the level of the allantoic-occipital junction. In all but one the character, distribution, and rate of myoclonus in limbs and trunk were unaltered. Complex spinal reflexes were observed and these spinal animals were able, without artificial respiration, to maintain adequate pulmonary ventilation for hours solely by myoclonic contractions. Additional acute transections were performed in animals in which the myoclonus resulted from virus inoculation directly into the spinal cord. In six cats infected in the cervical region, the myoclonic movements developed initially in the forelimbs and later involved the hindquarters. Midthoracic cord transection then abolished the myoclonus completely in the lower extremities but had no effect on muscular contractions in the forequarters Conversely, in seven cats inoculated into the midthoracic or lumbar spinal cord, myoclonus made its first appearance in the hind legs and subsequently in the forelimbs. In these animals acute high thoracic *transection abolished* the *movements in the forequarters, leaving those in* the hind limbs unchanged.

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Continued advances in the study of viruses in cells and animals have made the clinical virologist aware of the great difficulties of assigning etiological significance to a particular agent when it is isolated from the patient. This is particularly true of chronic infections in which an antibody rise no longer occurs and therefore gives no circumstantial evidence of the presence of virus. On the one hand, virus may be repeatedly isolated from apparently healthy organs, as witness the adenovirus from the adenoid *tissues obtained at operation*, *on the other hand,* classical *virus infections* such as chicken pox or herpes zoster, or both, have yielded an agent which kills cells and spreads from cell to cell in tissue culture, but which has not been recovered from the tissue culture fluid or from homogenates of the infected cells *Thus, virus may be recovered* in the absence of disease, or infectious virus may be absent in the presence of specific viral lesions.

In a series of young children, Rowe *et al.* (63) have shown that the virus of human salivary gland disease may be recovered repeatedly from mouth swabs over a period of two to five months and from 8 to 24 months after the infection originally produced antibody The virus was also present in the urine *as long as 15 to 24 months after neutralizing antibody had first* been demonstrated. Furthermore, virus was found only in those children

shown by any of the three techniques in less than 13 days after the vaccination. Significant neutralizing antibody then appeared in one at 13 days and another at 16 days. From then on antibody titers rose quickly in all and were maintained for indefinite periods thereafter. In determining antibody titer in individuals who were vaccinated in previous years, neutralizing antibody was found in many instances 20 years after vaccination, and in one case 40 years afterward without revaccination (82). In most cases the antihemagglutinins were not detectable after eight months and the complement-fixation antibodies were gone at six months. Revaccination produced a more rapid rise in antibody titer The antibody studies confirmed the difficulty of determining whether a response to revaccination is an allergic or an immune response. Of 15 individuals with an early response to revaccination, only 8 showed a substantial increase in antibody. In smallpox patients not vaccinated before the acquisition of the disease, variola-neutralizing antibody appeared after six days (83). In most cases the antibody response was good, but of 10 fatal cases the antibody response was low except in two in whom the response was high on the second and fourth days Antibody studies confirmed the smallpox etiology of several febrile attacks which occurred in contacts who developed febrile attacks without rash

Psittacoid viruses.—It has long been recognized that trachoma is a virus disease, and because of the morphological appearance of the inclusion, it has been believed to be a member of this group of agents A claim by Chinese workers (Chang *et al.*) that the virus may be cultivated in the yolk sac of chick embryos, seems to have been confirmed by Collier & Sowa (84) who isolated a second strain and who determined the pathogenicity of their cultured virus for man by the inoculation of the conjunctiva of a human volunteer who then developed typical trachoma Subsequently, Bell *et al.* have indicated that there are two antigenically different strains as tested by toxin neutralization (85).

The well-known group specificity of the complement-fixing and skin-testing antigens of this group of viruses was further studied by Hilleman *et al* (86) who suggested that species which are less virulent for man, such as the mouse or feline pneumonitis virus, might well be employed in the manufacture of the skin antigen used in testing for antibodies to all members of the group

The discovery (87) that the group-specific antigen is also present in a common bacterium, *Bacillus anitratum*, led to the hope that a simple and safe method of preparing an antigen for complement-fixation was at hand However, as pointed out by Matthiesen & Volkert (88) not every strain of the bacillus produces the antigen and the conditions necessary to obtain such an antigen consistently are not as yet known.

The transfer of these agents to man continues to be an occupational hazard of unknown measure. Two recent studies demonstrate this. When a case of psittacosis on a chicken processing farm was investigated, it was

of picking it up on blind animal passage, the well-recognized fact that passenger agents (mostly bacteria) have been recovered from this pathological tissue in the past makes the etiological significance doubtful.

Secondly, de Ruyck *et al.* (77) have, in a series of papers, claimed the isolation of an agent from human chorioepitheliomas. The agent has many similarities to the common ectromelia of mice and a mouse source of this virus has not been excluded

With increased evidence that leukemia of mice is caused by a virus, attention has naturally been directed toward human leukemias. Schwartz & Schoolman (78) suggested that leukemia is a response of the host to an "offensive agent" and obtained certain unusual reactions between guinea pig sera and brain tissue from mice which had been injected with leukemia cells. This led them to attempt to isolate a substance from the brains of leukemia patients which would produce leukemia in mice. In their published work they report the successful great acceleration of leukemia by extracts of the brain of people who have died from leukemia (79). This extract is positive in many but not all cases, and there seems to be no one type of leukemia which produces the effect. The extract loses its activity when heated for 45 min. at 65°C. An inquiry into the relation of previous infection with mumps in patients with malignant testicular tumors showed that 5 of 120 cases had previous orchitis 8 to 26 months before (80).

One must conclude that as yet there is no convincing evidence for viral etiology of human malignancy, and at the same time that much more work will have to be done before one may venture any conclusions in the matter.

Animal-Human Virus Relationships

Theobald Smith in his lectures on "Parasitism and Disease" (81) spoke of the aberrant parasite as "that adventurous element in the life of the parasite which led to new disease or death of the parasite" Virology was just beginning to find a place in medicine at that time and this may be taken as an impressive prediction of the present recognition of the relationship of animal reservoirs of virus infection to disease in man

Many of the animal reservoir-human disease relationships depend upon transfer by vectors However, this section will be limited to those which are transferred by direct contact.

Vaccinia; Variola—Despite the continued use of the cow pox virus derivative, vaccinia, for the production of immunity against smallpox, there has been little adequate direct measurement of the protective antibody against the smallpox virus (variola) which follows vaccination. Downie and his colleagues first developed a more accurate and standard measurement of these neutralizing antibodies. They then determined the response in man to vaccination by means of the variola neutralization test, and the development of antihemagglutinating and complement-fixing antibodies. In a number of students vaccinated for the first time no antibody was

likely that the next few years will see increasing complexity in the relationships and demonstrated transfer of these agents from animals to man and man to animals (99).

The role of Newcastle disease virus as a cause of disease in man has been confusing. It was first demonstrated by Burnet as a cause of acute conjunctivitis in man (100) and this syndrome is now well recognized. Subsequently there was confusion because of failure to recognize that human sera have high titers of non-specific neutralizing substances and must therefore be inactivated before results indicative of infection with this virus can be obtained However, with heated sera it has been shown that people with known infections develop antibodies to the virus Furthermore, chicken and laboratory workers exposed to the virus have a high incidence of antibodies. Yet, there are clearly individuals who give no adequate history of exposure who also have antibody to Newcastle. Furthermore, many mumps patients (though none who were vaccinated against mumps) have antibody to Newcastle disease virus (101). Also mumps virus may be used to absorb the antibody to Newcastle from human sera These results indicating a relationship between mumps and Newcastle were not apparent in studies on animals injected with one dose of virus (102) but did occur when animals were hyperimmunized (103).

One may conclude that infection of man with Newcastle disease virus does give rise to antibodies to the virus, but that perhaps half of the patients with mumps develop an antibody which crosses with the Newcastle virus.

A similar problem exists concerning the relationship of measles and distemper virus of dogs This relationship, proposed originally on a basis of general and cellular pathology, has been recently discussed in reviews by Rake (104) and by Black *et al* (105) The latter authors concluded that "the evidence of a common antigenic component in canine distemper and measles has been well established" Most of the data, however, have rested on the study of antibodies in man, either as parts of surveys or following attacks of measles However, Carlstrom (106) also studied the reaction of dogs inoculated repeatedly with dog-kidney-adapted measles virus and found no clinical signs following infection although neutralizing antibodies to both viruses appeared in the serum Monkeys were then inoculated with egg-adapted distemper, and two of the three so inoculated developed antibodies to both viruses. Finally, two congenitally defective children were inoculated with egg-adapted distemper and despite the lack of clinical illness, one developed a rise in antibodies over a previous low level to both viruses, while the other developed for the first time detectable antibodies.

A more recent report of Cabasso *et al* (107) seems, however, to establish the fact that neither dogs infected with chick-adapted distemper virus, nor chickens given multiple doses of measles develop antibodies to the opposite virus These negative data, however, do not contradict the premise of a general relationship

found that 9 of 70 chickens on the farm had antibodies, and a serologic survey of the workers who eviscerated the poultry showed 14 per cent to be positive (89). In another outbreak, 20 of 41 employees in a poultry processing plant developed symptoms one week after handling a shipment of 2500 turkeys from a supply farm (90) The highest attack rate in the people was among those unloading. A rise in complement-fixing antibodies to lygranum (lymphogranuloma inguinale) was detected in 13. The virus of "psittacosis" was isolated from frozen "dressed" turkeys and also from some live turkeys remaining at the farm.

Myxoviruses.—With the continuing isolation of viruses of the Influenza-Mumps-Newcastle Disease group, there is an increasing need for study of the antigenic relationships of the different members so that serological data from man may be correctly related to the specific virus causing the infection Undoubtedly, the classification of the agents themselves will also influence the classification of the diseases It is hoped that it will also increase our understanding Currently, three new members have been proposed for this group (91). Two were discovered as a result of the capacity of infected cells to be adhesive for red cells, and have been called hemadsorption viruses (92). The third is the croup-associated virus isolated by Chanock (93). All three are designated as *para*-influenza viruses because they seem to cause a disease in man somewhat similar to influenza. In the original communication (91) it was suggested that since one of the hemadsorption viruses is very closely related by complement-fixation to Sendai or Japanese hemagglutinating virus, the latter should also be included in the *para*-influenza group This would be consistent with the increasing evidence that Sendai virus causes widespread infection in man (94) and at the same time explain its closer relationship (morphology and hemolysis) to Newcastle and mumps viruses Thus, presumably the disease caused by it would be called *Para*-influenza instead of Influenza D

These relationships are discussed in detail here because the classification of this group now includes under the Influenza A title two new viruses (95, 96) isolated, respectively, from horses and from ducks, together with the well-known swine influenza and fowl plague viruses. Presumably, each of these animal viruses is sufficiently different from human influenza (by neutralization as contrasted with complement-fixation tests) that antibodies to other members of the group, such as the various recent strains of Asian influenza, will not cross by neutralization tests with these agents However, the exact role of swine influenza in past pandemics is still under study, and a recent outbreak of influenza in horses was attributed to a strain of influenza A very similar to Asian (97)

In addition to this, Abinanti & Huebner (98) indicate that there is a definite serological and cultural relationship between the strains of *Para*-influenza 3 isolated from man and several new viruses isolated from shipping fever in cattle. In this case they could not be differentiated by hemagglutination-inhibition or neutralization tests. It would then seem

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Further epidemiologic evidence that measles may be a source of antibodies to distemper is presented by Carlström who found that five of six children with measles in Iceland, where distemper is absent from dogs, nevertheless developed antibodies to distemper (108). Finally, Adams *et al.* (109) make the tentative suggestion that immunization with live avian distemper virus may have reduced threefold the susceptibility to an outbreak of measles in a state institution for the mentally retarded.

In addition to these animal-human virus relationships, it now appears that canine hepatitis may be a member of the adenovirus group (110), while Klein *et al.* report the isolation of an adenovirus from cattle (111).

SUMMARY

In writing this review we have considered areas of endeavor which are only partially developed. Common as viral infections of the respiratory tract may be, too little is known of their pathogenesis This was well brought out by review of the studies on Asian influenza. The startling symptomatology of infection of the central nervous system is now beginning to be studied by neurophysiological means. The presence of prompt antibody response to most virus infections has blinded us to the fundamental problem of chronic virus infections. Several of these are now being more thoroughly studied, and the problems of diagnosis will no doubt be similar to those of chronic brucellosis Finally, antigenic relationships between animal and human viruses are being found more and more frequently, and will add to the complexity of definition of human disease.

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INFECTIOUS DISEASE: BACTERIAL[1]

By Vernon Knight, M.D.
Laboratory of Clinical Investigation,
Institute of Allergy and Infectious Diseases,
National Institutes of Health, Bethesda, Maryland

Introduction

The field of bacterial infection in the past two years can be characterized more by the problems it has presented than by the progress which has been made. We seem to be in a lull after the period of turbulence which marked the first saturation of our population with antibiotics. No antibacterial drugs of exceptional and unique properties were developed and no great advances in the treatment of infection occurred. Diseases of greatest interest were those caused by staphylococci and Gram-negative bacilli, organisms which, under normal conditions, are benign inhabitants of the skin and mucous membranes of man, but which either naturally possess, or soon acquire, antimicrobial resistance. The infections caused by these organisms, their frequency, epidemic tendencies, pathogenesis, treatment, and explanations for their current prevalence, are the principal problems which are described in this review. We have formed the opinion that these problem infections are not going to be effectively controlled by presently available antimicrobial therapy, and that their alleviation must probably await the discovery of new approaches to treatment Some encouragement for such developments may be found in several new concepts of infection and its treatment included in the following review

Staphylococcal Disease

Apprehension about staphylococcal infection in hospitals has resulted from the fact that the disease is frequent, often fatal, and usually caused by strains resistant to antimicrobial drugs. It is certain that the high prevalence of resistant staphylococci in hospitals is an effect of the use of antimicrobial drugs, and the possibility has been considered that the present problems with infection may, in part, reflect a greater than usual capacity of these staphylococci to cause illness.

According to Langmuir (1), staphylococcal infections in hospitals may be separated into four general categories: (*a*) pyoderma neonatorum and its complications; (*b*) mastitis and breast abscesses in postpartum mothers; (*c*) surgical wound infections; and (*d*) infections superimposed on other diseases in debilitated patients. These latter infections occur characteristically on medical wards.

In 1958 the American Hospital Association (2) and the Joint Commission on Accreditation of Hospitals (3) took formal notice of the problem

[1] The survey of the literature pertaining to this review was concluded in July, 1959

by issuing bulletins to hospitals recommending procedures for the prevention and control of staphylococcal and other infections The bulletins were published following discussions of staphylococcal disease at conferences sponsored by the American Public Health Association (4 to 7) and the American Medical Association (1, 8 to 14). Subsequently, the Communicable Disease Center, U S Public Health service, with the National Academy of Science and the National Research Council, sponsored two meetings (15, 16) in which representatives of numerous branches of medicine met to consider the problem.

The results of the various conferences in terms of development of effective control measures were few. A summary prepared after the conference called to consider environmental aspects of staphylococcal disease (16) is indicative of present thinking on the problem. It was concluded that staphylococcal disease is endemic in hospitals, that the epidemiology is incomplete, but control measures must be started while knowledge is accumulating, and that criteria for housekeeping practices and air conditioning design as related to control of spread of infection are virtually non-existent.

It could be added that current therapy of serious infections is relatively inadequate and an important deficit exists in our knowledge of the microbiology and immunology of the disease. Recent reports, however, have provided some additional useful information One of particular interest is a comprehensive review of literature on *Staphylococcus pyogenes* by Elek (17).

Clinical studies.—Yow and her associates (18), in a report on 204 cases of staphylococcal infection among children in a hospital, stress the fact that the disease is apt to disseminate in infants, with resulting high mortality, especially in those born prematurely. They found a mortality rate of 43 per cent among prematures, in contrast to a death rate of only 12 per cent among older children. They felt the rapid dissemination in infants was indicative of poor host resistance and found evidence of better results in treatment when bactericidal agents such as bacitracin and kanamycin were used. Beaven (19) suggests that staphylococcal infection may be currently on the increase, based on his observation of five cases of staphylococcal peritonitis in children in a recent two-year period, in contrast to an absence of such cases in that institution and elsewhere for several years previously The disease is characterized by a tender, distended, "silent," abdomen. No case was observed before the fourteenth day of life, which finding presumably indicates the presence of protective maternal antibody Several of the cases were fatal. Another manifestation of staphylococcal infection is staphylococcal spinal meningitis, described by Studdert (20) He considered that some cases represented local extension from epidural abscesses and stressed the localized spinal involvement. Treatment consisted largely of intrathecal and systemic penicillin and only one of seven patients died. Watkins (21), in considering staphylococcal pneumonia, described frequent pleural complications of the disease, especially empyema. He reports that closed drainage

may often obviate rib resection and other general surgical procedures. These studies stress the seriousness of staphylococcal infection in infants and its tendency to chronicity and metastasis at all ages.

Jawetz (22) has recently contributed important information concerning the antimicrobial therapy of staphylococcal infection. As an example of the benefit of his method of treatment, he described the recovery of 11 patients seriously ill with staphylococcal infection His method is to test the infecting organism *in vitro* against most of the available antimicrobial drugs, singly, and in combinations of as many as six different agents, to determine which is the most rapidly bacteriocidal. Within limits of tolerance and toxicity, the best combination is used in treatment The course of illness of the patients was long and relapses were frequent. Drug combinations were changed at intervals of a week or so; often three to five different drugs were administered simultaneously. While these were not consecutive cases, the seriousness of their illness suggests that these are especially good therapeutic results. Jawetz' method is a rational approach to treatment of a disease which is one of the greatest present challenges in the field of antimicrobial therapy.

In additional comment, he states that he has observed slight or no improvement in cases of staphylococcal infection with penicillin-resistant strains when high doses of this drug were given alone. This comment was made in connection with the recommendation of Fisher (23) and Petersdorff (24) that penicillin in massive doses should be employed in cases of staphylococcal infection caused by organisms partially resistant to penicillin, which was in contrast to the opinion expressed by Finland (25) that penicillin alone, or in combination, was not beneficial against infection which is moderately or highly resistant to the drug. The reviewer's experience is in agreement with that of Finland

The question of whether or not it is advantageous to treat boils with antimicrobial drugs often arises Scott & Waterworth (26) have provided useful information on this subject by a careful description of a long series of patients with boils treated with erythromycin or antibiotic E129, and compared with results obtained on a similar group who were given only time-honored conservative therapies They found that patients who received antibiotics recovered more promptly, had less tissue destruction, and were bacteriologically negative in a considerably shorter time than were the untreated patients. Drug treatment did not exceed one week and resistant strains were not found The decision to use antimicrobial drugs in the treatment of boils may depend on many considerations, but this evidence of benefit from treatment *may assist in making the choice*

Fatal bacteremia with staphylococci has developed in patients receiving steroid therapy for a variety of illnesses such as disseminated lupus erythematosus, hemolytic anemia, leukemia, and exfoliative dermatitis. Such experiences and the knowledge that steroids may cause depressed resistance to infection and interfere with antibody production (27), have discouraged their trial in the treatment of staphylococcal infection Nevertheless,

Bertrand-Fontaine & Cheymol (28) have reported their own and others' experiences in which rapid defervescence and improvement followed corticosteroid treatment in patients ill with staphylococcal bacteremia A similar result has recently come to the attention of the reviewer. These few observations are obviously an unsatisfactory basis for determining a therapeutic program, but the acceptance of corticosteroid therapy in acute tuberculosis (in association with antituberculosis drugs), and its apparently favorable effect on some other infections (29), indicates the possibility that there exists a property of corticosteroids which might be employed to better advantage in the treatment of infection if it were better defined.

Epidemiology—To diminish resistance to penicillin in the staphylococcal flora of a hospital, Barber *et al.* (30) abandoned the use of this drug and substituted erythromycin, chloramphenicol, and novobiocin in various combinations. Combinations were used to retard the development of resistance known to follow the use of erythromycin and novobiocin alone. From observations of resistant strains produced during a six-months' study, it was determined that there was evidence of cross-resistance between erythromycin and chloramphenicol, so that at times the use of one of the drugs would result in strains resistant to both drugs. No such effect was disclosed with novobiocin. *In vitro* studies later confirmed the existence of cross-resistance between the two drugs It appears that the use of this combination should be avoided if possible.

White *et al.* (31) have shown that the administration of penicillin and, on a later occasion, tetracycline, to patients in a mental hospital in whom resistance of staphylococci to these drugs was very low, was nevertheless followed by a rapid increase in staphylococci resistant to the respective agents. They suggested that programs of restriction of use of penicillin and tetracycline in order to restore their therapeutic effectiveness in staphylococcal infections, were not likely to succeed

While it was admitted earlier that methods for the control of spread of staphylococcal infections were inadequate, some outbreaks appear to have been terminated by the identification and removal of carriers of the epidemic strain from the area (5, 6, 32, 33). Such efforts are not invariably successful and Williams *et al* (34) have described an outbreak in which vigorous and well-conceived efforts to eliminate carriers and improve environmental sanitation were not successful They felt that infection was so widespread in their hospital that the measures described could not be successful unless patient loads were reduced to the point where more extensive isolation of cases and carriers could be achieved This, they felt, might prove to be impractical

It was implied in these authors' reports that when the strain of staphylococcus causing an outbreak is widely disseminated among patients and personnel of a hospital, the control of infection may not be possible short of closing the institution or greatly reducing its services. In certain geographic areas the need for hospital facilities is so urgent that only under the greatest pressure can patient-care programs be restricted Outbreaks among adult

patients are less likely to become severe enough to require closing the hospitals but in nurseries this expedient is not infrequently necessary. An example of such an outbreak was described by Timbury *et al.* (35), in which a severe nursery outbreak was terminated by this procedure.

Laboratory—The diagnosis of staphylococcal infection by serologic tests has been attempted many times and, although immune responses to the infection do occur, for various reasons, successful tests have not been developed. A more promising effort in this direction has been described by Towers & Gladstone (36). Their test is based on the demonstration of an antibody to a non-hemolytic leukocidin produced by coagulase-positive staphylococci. This leukocidin was first described by Van der Velde in 1894 The leukocidin produces characteristic degenerative changes in human white cells *in vitro* which can be prevented by the presence of the specific antibody in the patient's serum (37) Although only about one-half of a series of cultures of coagulase-positive staphylococci were found to produce this substance *in vitro*, 82 per cent of 83 cases of staphylococcal infection showed increased antileukocidin titers. In contrast, titers of antialphahemolysin, another test for staphylococcal infection, were elevated in only 33 per cent of the cases. Among cases of non-staphylococcal infection, antileukocidin was increased in 17 per cent while none showed increase in antialphahemolysin. It was postulated that some patients with non-staphylococcal infection might also have additional small infections caused by staphylococci to account for the cases indicating apparently false positive reactions. This test may prove valuable in the diagnosis of staphylococcal infections, but it is also of importance in that it is one of the few examples of consistency between human infection resulting from staphylococcus and an immune response to it.

A valuable development in studies of the immunology of staphylococcus is a procedure which causes ascites in mice rich in staphylococcal antibody. Lieberman and her colleagues (38) have found that Freund's adjuvant (paraffin oil, a solubilizing agent and heat-killed *M butyricum*) complete, or lacking *M. butyricum*, when combined with preparations of staphylococcal cells and injected intraperitoneally will, within a few days, cause ascites in mice with a high titer of staphylococcal agglutinating antibody (1·2000 to 1 4000) Several ml of fluid may be withdrawn at each of several aspirations. This methodology provides a convenient means of producing relatively large volumes of staphylococcal antibody and may also make for rapid comparisons of different antigens of the organism

By observing colony growth in soft agar, Finkelstein & Sulkin (39) have shown that coagulase-positive colonies of staphylococcus grow in a compact manner in human or rabbit plasma, whereas coagulase-negative strains grow in loose clumps They refer to this as a "colony compacting" reaction Based on studies made of rabbits injected with a staphylococcal vaccine, it appeared that the degree of compacting might be a measure of antistaphylococcal antibody. This reaction is apparently similar to the dense clumping of coagulase-positive colonies which are often seen in diagnostic blood cultures

of patients with staphylococcal bacteremia. Szeto & Halick (40) have shown that production of coagulase is supported by a dialysate of heart infusion broth, a substance they believe to be a polypeptide. In another study of nutritional requirements for enzyme production, Steinman & Murtaugh (41) have devised a synthetic medium containing 14 amino acids which supports moderate growth and considerable penicillinase production of a penicillin-resistant culture of staphylococcus. However, if isoleucine, one of the 14 amino acids, is omitted from the medium, growth but no penicillinase formation occurs. Leucine, serine, and valine act in a similar way but to a lesser extent.

One hoped-for result of the search for methods to control enzyme production of staphylococci would be the development of ways of employing them to modify virulence or drug resistance in human infections. Sallman & Streitfeld (42) have approached the problem of penicillinase production of staphylococci directly by seeking compounds which inactivate penicillinase, hoping that their administration might restore the effectiveness of penicillin *in vivo* against staphylococci. They found that trypsin, chymotrypsin, quinacrine, and chloroquin had the greatest effect of the several compounds tested *in vitro*. No studies are yet available of *in vivo* tests.

Hanka & Lockhart (43) have found a strain of *Staphylococcus aureus* which is inhibited by diethylstilbestrol, but not by several closely related compounds Using the organism in a bio-assay, it was possible to detect drug concentrations of only a few micrograms. It is not known how widely susceptibility to diethylstilbestrol is distributed among staphylococci, nor is it known what relationship exists between the endocrine effects of the compound and its antibacterial action, but it would be interesting to consider the possible application of the substance in staphylococcal infection.

Pyelonephritis

In the two years since pyelonephritis was last considered in these volumes, important new information has appeared concerning several aspects of the disease The modern criteria for the diagnosis of pyelonephritis were described by Weiss & Parker in 1939 (44), based upon clinical and autopsy studies. Recently, Merriam *et al* (45) have proposed criteria for the microscopic diagnosis of pyelonephritis based on observations of renal biopsies from hypertensive patients Insofar as they are comparable, there is agreement on the criteria set forth in the two studies. In addition to chronic pyelonephritis, Merriam and his colleagues distinguished acute and healed lesions of the disease. Their criteria for microscopic diagnosis were: chronic pyelonephritis—irregular scars, dilated tubules containing colloid, protein casts, and plasma cells mingled with other leucocytes in the stroma. The presence of plasma cells was considered essential to the diagnosis; healed

biopsies from about 1700 patients with hypertension gave evidence of pyelonephritis, predominantly of the chronic variety. It was further found that while nearly all of these patients had arteriolar sclerosis, it was more severe in the patients with pyelonephritis. In another recent study by Baumanis *et al.* (46) of 900 consecutive autopsies in a chronic disease hospital, 20 per cent of the cases were found to have pyelonephritis but only 10 per cent of the cases of pyelonephritis had a history of hypertension. In a study of 977 autopsies from U. S. Veterans hospitals (47), pyelonephritis was found to be present in 14 per cent of the cases, although no statement was made concerning the occurrence of hypertension.

Thus, pyelonephritis and hypertension co-existed in only about 10 per cent of a series of cases of hypertension and to the same extent in a series of cases of pyelonephritis. This low rate of coincidence of the two diseases [illegible]

ever cause-and-effect relationship exists between the two diseases.

In this connection, Riley & Knight (48) have described increased frequency of hypertension in patients with chronic, severe paralytic poliomyelitis. The hypertension often first appeared in the acute phase of the disease and continued throughout many months of the chronic paralyzed state. In seeking a cause for the hypertension, they found a negative correlation between the occurrence of pyelonephritis and hypertension in these patients. In a more recent report, Shapiro *et al.* (49) failed to find a development of significant hypertension in rats with severe experimental pyelonephritis which they observed for periods as long as one year.

There is some agreement that the bacteria which cause pyelonephritis originate in the gut. A number of routes have been described by which microorganisms may be carried from the intestinal tract to the kidney to cause infection. In a recent discussion of the question, Gorrill (50) suggests that spread through urine to bladder and up the lumen of the ureter to the pelvis of the kidney, or by the hematogenous route, are the most popular current alternatives. Gorill believes the hematogenous route to be the most important, but he recognizes that the inability of intravenous injections of *Escherichia coli* to cause pyelonephritis unless there is concomitant obstruction to urinary flow indicates a more complex chain of events. Talbot (51) agrees that pyelonephritis may be hematogenous in origin, but when it follows infection in the bladder, he believes it may ascend to the kidney through the subepithelial areolar tissues common to the bladder, ureter, and kidney pelvis. Moreover, once the lower ureter has become inflamed obstruction to the flow of urine may occur, adding this important contributing factor to the pathogenesis. This thesis has much to recommend it.

New information concerning the pathogenesis of pyelonephritis has emerged from the findings that the renal medulla and cortex react differently to inocula of bacteria. In 1956, Gorrill (52) discovered that following intravenous injection, the number of bacteria in the medulla of a kidney

with ureteral obstruction increased logarithmically without delay, while in the cortex there was a lag period of about 90 min. before logarithmic increase began. These changes continued to occur in the medulla for several days after the release of the ureteral obstruction, whereas the renal cortex promptly returned to its normal high resistance to infection. Thus, the ureteral obstruction necessary to produce most kinds of experimental pyelonephritis only briefly increased the susceptibility of the cortex to infection in contrast to a prolonged state of increased vulnerability of the medulla.

Further evidence of the greater susceptibility of the renal medulla to infection was reported by Beeson, Rocha & Guze, in 1957 (53) and, in later reports, by Rocha, *et al.* (54) and Guze & Beeson (55). Instead of initiating ureteral obstruction as a condition necessary to produce experimental infection, they damaged the renal cortex and medulla by electrocautery. Following this the animals were inoculated intravenously with a culture of *E. coli*. It was found that 85 per cent of those animals with medullary lesions developed acute pyelonephritis at the sites of injury while none developed in renal cortices similarly damaged. Bacterial counts made of medullary tissue showed a prompt increase and, in agreement with Gorrill's findings (52), a delay of growth in the cortex. Furthermore, bacterial counts in the cortex never reached the high values of those in the medulla. Guze & Beeson (55) also found that fewer than 10 bacilli inoculated directly into a damaged medulla would initiate infection while 100,000 or more were usually required to accomplish the same result in the cortex. The medullary lesions were described by these investigators as "intrarenal" hydronephrosis, and it was their concept that tubular obstruction was in some way causally related to the increased susceptibility of the renal medulla to infection

No recent significant advances in the treatment of pyelonephritis have appeared Jackson *et al* (56) have described recent therapeutic experiences which provide criteria of the best in the current therapy of this disease. They found that about 70 per cent of patients with acute manifestations responded well to antimicrobial therapy but that relapse occurred in more than one-half of a group of such patients within a few months. Among chronic pyelonephritis patients only about one-fourth responded to treatment, but it was pointed out that prolonged illness was in part caused by non-infectious disease. These investigators treated patients with acute pyelonephritis for ten days to three weeks and chronically ill patients for as long as six weeks.

Based on more or less an empiric experience, there has been an increasing belief that urinary tract infection should be treated for a long period of time. While a shift to a microbial species resistant to antibodies may occur, thus diminishing the benefit of treatment, a number of observers feel that the prospect of a more durable cure from long treatment justifies its use (57).

Evaluation of therapeutic agents in urinary tract infection is difficult because of the frequent presence of underlying urologic disease which modifies

the course, and because spontaneous remissions occur. As a consequence, estimates of the effect of antimicrobial therapy should place strong dependence on studies of the bacterial flora of the urine before and during treatment, and for a minimum of several weeks thereafter. In this way accurate laboratory information can be secured to supplement clinical observations. A study which reveals the value of this type of surveillance was reported by Dock *et al.* (58) who discovered that although clinical remission was prompt, 8 of 30 patients with non-obstructive acute pyelonephritis had bacteriuria for several weeks after leaving the hospital. *E coli* was isolated from seven of the patients initially and from six subsequently. Treatment at first admission was given for 7 to 18 days but most often for only one week. Retreatment was given for a similar period. It seems certain that patients in whom persistent infection is found, whether symptomatic or not, are at great risk of chronic pyelonephritis and special effort should be made to detect them so that sufficient treatment may be given.

Better than the treatment of pyelonephritis would be its prevention. The greatest application of preventive measures lies in giving protection from renal infection during urologic procedure or catheterization It is almost a general practice to give "prophylactic" antibiotics before surgical procedures and during periods when catheters are in place. There is no uniform agreement as to the benefit or lack of benefit of such measures Recently, however, Weyrauch *et al.* (59) have approached the problem through studies of acute pyelonephritis in animals They found that the administration of tetracycline to rabbits inoculated with *E. coli* in the presence of obstructed ureters prevented the development of pyelonephritis which occurred in almost all control animals not given antibiotics. The drug was given one day before inoculation with bacteria and continued for five days. In another [illegible]

biotic treatment before certain urologic surgical procedures.

Bacterial Pneumonia Complicating Influenza

The pandemic of Asian influenza struck many large cities in the United States almost simultaneously in the Fall of 1957 and, to a lesser extent, the following Spring In New York (60, 61), Boston (62), New Haven (63), Cleveland (64), and New Orleans (65) studies were made of severely ill or fatal cases, in many of which bacterial pneumonia was a complication In addition to bacterial pneumonia, there were cases in which the diagnosis of pure viral influenzal pneumonia was made, based on negative cultures for pathogenic bacteria, evidence of influenza viral infection, and proof of pneumonia on clinical or pathological grounds Not infrequently, both diseases were recognized in the same patient. The pneumococcus was the most frequent cause of bacterial pneumonia but most of the "fatal" cases of bacterial pneumonia were caused by staphylococci. In addition, many fatalities occurred among those with pure viral influenza The pneumonia

afflicted all age groups and many fatal cases occurred after only a brief illness among young, previously well persons, in a manner reminiscent of the devastating 1917–1918 pandemic. There was considerable agreement in the findings that pregnancy, rheumatic or other cardiac disorders, and chronic pulmonary disease increased susceptibility to pneumonia and death from influenza. Treatment of pure influenzal pneumonia was largely supportive with emphasis on oxygen therapy to prevent suffocation. It was not notably effective Complicating pneumococcal pneumonia usually responded to treatment while those with staphylococcal involvement often ran a fatal course. Martin *et al.* (62) felt that cases of all kinds of pneumonia occurring in an influenza epidemic should be promptly treated as staphylococcal in origin, since the disease often progressed too rapidly for definitive pretreatment diagnostic studies.

Bacterial Endocarditis

Dormer (66) described data which reveal a decline in mortality from endocarditis in England and Wales from about 800 annually in 1944 when penicillin first became available to little more than 300 in 1952, with little further reduction subsequently. In a review of cases at a British hospital in the period 1945 to 1956, he found that about two-thirds of the endocardial infections in 82 cases were caused by *Streptococcus viridans* and only five were caused by staphylococci, two of which followed the only two mitral valvulotomies described in the series. The immediate mortality of the whole series was 25 per cent and there was a recurrence rate of 18 per cent. In the United States, Hoffman *et al.* (67) cite reports of an increasing incidence of staphylococcal endocarditis in recent years and, in accord with this trend, describe 26 of 40 cases of endocarditis arising after cardiotomy for acquired heart disease caused by staphylococci These were about equally divided between coagulase-positive and coagulase-negative strains. The mortality rate of patients with staphylococcal infection was 50 per cent.

Finland (25) has recently reviewed the present treatment of endocarditis It is his experience also that about 50 per cent of staphylococcal endocarditis cases succumb In addition, he notes that mortality is high in the cases of endocarditis in which blood cultures are repeatedly negative He expects as high as 90 per cent recovery, however, from infection with penicillin-sensitive organisms, such as most strains of *Streptococcus viridans* Staphylococcal endocarditis may be treated by Jawetz' method *S. viridans* and enterococcal infections may be treated with streptomycin and high doses of penicillin for three to several weeks, depending upon the response Enterococcal infections usually require longer treatment.

Rheumatic Fever and Streptococcal Infection

Of particular recent significance in problems of streptococcal infection and rheumatic fever is the preliminary report by Mortimer, Vaisman *et al.* (68) of a probable reduction in valvular heart disease among acute rheumatic fever patients treated with penicillin Treatment was begun within

a few days of the onset of illness and continued for six weeks. At this time, these patients and matched controls previously not treated with penicillin, were given repository penicillin treatments for one year. Studies at this time revealed that only 6 of 29 patients receiving penicillin had persistent organic murmurs, while 15 of 29 controls had such murmurs. This degree of difference was demonstrable only by removing from consideration patients in control and treated groups in whom fixed valvular disease was considered to exist before the initiation of treatment

Numerous comparisons between controls and treated patients gave no evidence that penicillin in any way alleviated the acute manifestations of the disease The authors comment that the apparent described effect of penicillin on valvulitis may indicate a different pathogenesis for this part of the disease, possibly a direct effect of the streptococcal infection. Undoubtedly, verification of these studies will continue, but until such findings are available established dicta should be followed to employ chemoprophylaxis in cases where there is risk of rheumatic fever, to detect it early when it occurs, and to treat it vigorously and sufficiently when it is identified

It should be recalled that only a small percentage of cases of acute respiratory infection are caused by group A streptococcal infection, and that few of those of non-streptococcal origin require treatment A British practitioner (69) withheld antimicrobial treatment in a series of cases of tonsillitis and other acute respiratory infection until definite indications for it were established He finally treated 25 per cent of the group. Rheumatic fever and other complications were not discovered in either group and it was considered that treatment is not needed in many of these patients

It has long been appreciated that rheumatic fever is more prevalent in low income groups In New York City (70), studies have now shown that a decline has occurred in rheumatic fever recurrences among children of school age from about one in four before 1944 to one in seven since then The improvement was attributed to better living conditions prevailing recently and not to the use of antimicrobial drugs

Interesting new information on the effect of penicillin on streptococcal immunity has been provided by Jannson & Klemola (71) in a study of second attacks of scarlet fever after penicillin treatment. Earlier data indicated that no more than two per cent and usually somewhat less than this number of untreated patients experienced second, late attacks of scarlet fever in periods of observation of five to nine years They found, however, in a study of 7837 cases in Helsinki which were treated with penicillin, that second attacks at least three months following the first occurred in 5 per cent of the cases The second attacks were most frequent among patients whose penicillin treatment was begun early in the illness

Treatment of Bacterial Infections With Gamma Globulin

A recent development in studies of bacterial infection has been the observation that gamma globulin is active against infections produced by certain bacteria which cause human infection Cameron, in 1947 (72) and

1949 (73), demonstrated these effects in experimental veterinary bacterial infections using gamma globulin from animal sources. Sonea *et al.* (74) in 1956 and, later, Fisher *et al.* (75), employed gamma globulin of human origin in treating experimental infections in mice. The beneficial effect appeared to result from the content of specific antibacterial antibody since it could be removed by adsorption (76, 77) and its potency varied widely among various bacterial species (75). Thus, doses of 3 to 12 mg. per kg. of body weight protected 50 per cent of infected mice against staphylococcal infection and approximately 50 mg per kg. protected them against *Pseudomonas* infection. More than 100 mg. per kg. were required to protect 50 per cent of mice against *Proteus*, *E. coli*, streptococcal, pneumococcal, *Salmonella*, and certain other bacterial infections. In an average man, 40 to 50 ml. of commercially available gamma globulin constitutes a 100 mg per kg. dosage, while only a few milliliters would be required for the 10 mg./kg. dose.

Investigations (77 to 80) have also shown that gamma globulin enhances the activity of chloramphenicol, ristocetin, and tetracycline in experimental animal infections. The enhancing effect as most evident against infections with staphylococci, streptococci, and *Pseudomonas*. Several observers noted that there was variation in the effect of different lots of gamma globulin.

Stimulated by these reports a number of investigations have now been made of the effect of gamma globulin in human bacterial infection (81, 82, 83). In most cases, antibiotics have been used with gamma globulin. Infections from staphylococci were most frequently treated, especially osteomyelitis, but cases of *salmonella* infection, tuberculosis, and pyelonephritis were included The uniform result was that two-thirds to three-fourths of patients showed excellent responses to treatment. On a number of occasions, this followed failure of antibiotic therapy alone All of the cases described had approximately normal values of circulating gamma globulin before treatment and these were not appreciably changed by it. Doses varied from 20 to 60 ml per week Treatment failures were associated with retained sequestra, mixed infections, or microorganisms resistant to the antibiotics used. *Salmonella* infections were notable for their lack of improvement with the treatment.

Most cases of infection so far described which have been treated with gamma globulin are complicated ones which have failed to respond to other therapies Evaluation of the beneficial effect is necessarily highly subjective. Nevertheless, the detailed reports provided lead to the definite opinion that gamma globulin, especially when used with antibiotic treatment, has definite therapeutic activity in bacterial infections, especially in those caused by staphylococci

Prevention of Infection in Chronic Pulmonary Disease

A pressing problem in present-day practice is that of bronchitis and pneumonia which complicate emphysema, bronchiectasis, and other chronic

pulmonary diseases. Antibiotics have been used liberally to prevent these occurrences but little definitive evidence has been obtained as to their benefit Cherniack *et al.* (84) have recently studied the problem of the effects of several drug regimens given continuously to a large number of patients for several months under conditions which permit quantitative evaluation. They found that patients treated with tetracycline had significantly fewer lower pulmonary tract infections than those who received placebos. Penicillin and oleandomycin together showed some benefit, while penicillin treatment alone was indistinguishable from placebo treatment. The relatively large doses of drug, 2 0 gm. daily for tetracycline and correspondingly large for the others, were not complicated by severe intolerance or toxicity. Pulmonary function did not change in the patients during treatment, nor was the volume of sputum production altered, even in patients who showed a reduced frequency of infection. These studies demonstrate the value of preventive treatment against superimposed pulmonary infection, but the current cost of several dollars daily for its use will unfortunately preclude its wide application.

Pseudomonas Septicemia

It is difficult to escape the impression that *Pseudomonas* septicemia is more frequent now than a few years ago. It is certain that in institutions which care for large numbers of patients with leukemia and cancer the disease is relatively common (85) On the leukemia service at the National Institutes of Health, it occurred in 13 patients in a 20-month period of observation Twelve of the patients died. During this time, there were 16 cases of staphylococcal bacteremia on the same service, but only four died. *Pseudomonas* septicemia is a fulminating illness, often associated with hypotension, jaundice, cardiac arrhythmias, and fever. The median survival time after the first positive blood culture was only 4.0 days among 23 cases. Among various possible provocative factors studied, it was found that antimicrobial therapy with broad spectrum agents and treatment of leukemia with antimetabolites were frequently associated with *Pseudomonas* septicemia The fact that 22 of a total of 23 cases died, indicates that the circumstances of its occurrence and the ineffectiveness of present treatment give the disease at present a nearly hopeless prognosis

Toxicity of Antibiotics

There has been little reason to suspect that broad spectrum antibiotics have appreciable renal toxic effects. Farbat *et al* (86), however, have measured serum concentration of some of these agents in patients with failing renal functions and have found them to be greatly elevated In further study of the problem they administered large doses of tetracycline derivatives and chloramphenicol to rabbits. They found that intraperitoneal doses of drugs sufficient to produce serum concentrations of 20 µg per ml.

of tetracyclines or 40 μg. per ml. of chloramphenicol resulted in uremia within 7 to 10 days. With increasing nitrogen retention, blood concentrations of the antibiotics increased rapidly. The doses given to the animals were greatly in excess of those usually given to man However, the pronounced increase in antibiotic concentrations with renal insufficiency suggests that average doses could accumulate in patients with uremia and cause further renal damage.

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√GASTROINTESTINAL DISEASE: PORTAL HYPERTENSION[1]

By Charles G. Child, 3rd, M D.[2]
The Department of Surgery, University of Michigan Medical Center, Ann Arbor, Michigan

In 1942, Frank Mann (1) characterized the circulation of the liver as "a subject so old that it is almost buried beneath its own literature." Portal hypertension, related so directly to the hemodynamics of hepatic circulation, appears well on its way to similar interment. The publications of the last year or two on this subject have been numerous. In addition to a large number of conventional scientific articles, textbook chapters have been rewritten and enlarged, one full size book (2) is available, two outsized monographs (3, 4) have appeared, and one excellent symposium (5) has been reported Much valuable information on portal hypertension is also available in a variety of texts on liver disease (6, 7, 8).

Diagnosis

Judged from the number of reports (9 to 13), percutaneous splenoportography has been enthusiastically accepted as an important aid in the diagnosis of portal hypertension Figley (14) has reviewed his own experience with this venographic technique and surveyed that of others He lists technical simplicity, local anesthesia (except in children), and small doses of sodium acetrizoate (Urokon Sodium) as the advantages of the procedure Figley considers the hazard of intraperitoneal hemorrhage minor (about 2 per cent) and the information obtained relevant to the site of obstruction to portal blood flow accurate and significant Panke (15) working in Rousselot's department of surgery has recorded his group's experience with 266 percutaneous splenoportograms They conclude that these provide reliable information on the presence and nature of portal obstruction Splenoportography is particularly valuable as a diagnostic technique when combined with splenic manometry Inherent dangers were bleeding (2 per cent) and pyogenic infection (2 per cent), a failure rate of 5 per cent occurred. Also emphasized is the usefulness of the procedure in evaluating postoperative patency of end-to-side and side-to-side portacaval shunts

A variation on splenoportography in portal hypertension is costal interosseus venography (16, 17, 18). Here the marrow cavity of the lower left ribs is injected with a radiopaque substance and films of the lower chest

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and upper abdomen obtained. The normal costal, azygous, and hemiazygous venous systems are distorted in portal hypertension. Sufficient experience with this method is not yet available, however, to rival percutaneous splenoportography in the diagnosis of portal hypertension.

Wedged hepatic venous pressures, continue to be used widely in the diagnosis and study of portal hypertension (19, 20, 21). Many have shown that pressures obtained by this method are high where portal hypertension is caused by cirrhosis of the liver, and normal or but little elevated in portal thrombosis. When measurements are made in the hepatic venule simultaneously with those in the splenic pulp and splenoportograms are obtained, valuable information on total hepatic hemodynamics becomes available. In addition to its manometric usefulness, samples of blood drawn from within the liver provide important data on hepatic blood flow and metabolism. For instance, Castleman *et al.* (22) have shown that portal pressure rises significantly in some patients with cirrhosis after ingesting meat but not after glucose. This phenomenon may find application in the medical management of patients with portal hypertension

Medical Treatment

Attention continues to be accorded to medical treatment of portal hypertension as a method of lowering portal pressure and preventing variceal hemorrhage. Leevy and his associates (23) have shown that the portal pressures (wedged hepatic vein and intrasplenic pulp) of patients with mild to intermediate cirrhosis may fall from hypertensive levels of 35 or so centimeters of saline to as low as 10 to 12 cm of saline after two to three months on carefully controlled dietotherapeutic programs This decrease in pressure achieved by medical treatment alone was associated with both clinical and biochemical improvement and with an increase in hepatic blood flow; in three patients varices subsided completely Patients with advanced cirrhosis, however, failed to derive such significant benefit from treatment. Here, falls in pressure from 35 to 25 cm. of saline were all that could be anticipated.

Danowski (24), writing on the usefulness of low-sodium diets, emphasizes that in cirrhosis with ascites the success of sodium restriction in achieving clinical improvement is often governed by maneuvers designed to improve renal excretion of sodium. A variety of measures may induce diuresis in some patients with cirrhosis but in most permanent remission from ascites does not appear unless underlying improvement in hepatic function follows Blakemore & Voorhees (25) have again emphasized protracted medical treatment prior to portal decompression for patients manifesting significant degrees of hepatic decompensation.

Surgical Treatment

By far the largest number of recent articles on portal hypertension have come from surgical clinics These record in detail individual experience and tribulation in preventing variceal hemorrhage by portal decompression.

There is unanimous agreement that shunts of good size which remain patent fully protect patients with intrahepatic or extrahepatic block from recurrent hemorrhage. There is less agreement, however, concerning the capacity of portal decompression to prolong survival of patients with cirrhosis beyond that of the natural history of the disease.

For the most part, these articles all reflect the major handicap of portal decompression today, namely, that of varietal prejudice in selection of patients for operation. Each carefully suggests in one way or another that ultimately random selection for surgical or for medical therapy will have to be introduced before the true answer to prolonged survival after portal decompression is achieved As yet, no one has arrived at this difficult goal with complete satisfaction.

Pending Utopia, Ludington (26) thoughtfully reports an experience with 158 patients with esophageal varices He believes that varices account for one-third of major upper gastrointestinal hemorrhages and three-fourths of the deaths from this cause. He believes that in 90 per cent of patients with portal hypertension this is caused by cirrhosis of the liver and in 10 per cent by portal thrombosis. He also believes that the development of varices or failure thereof concerns the fortuitous presence or absence of congenital portacaval shunts whereby victims of cirrhosis decompress their own portal systems Medical therapy improves hepatic function in those with and without varices, but is ineffective in preventing or controlling hemorrhage He records a group of nine patients subjected to decompression, seven portacaval and two splenorenal There was one operative death Ludington suggests that the side-to-side shunt may ultimately prove safer than the end-to-side

Linton (27) again reviews his experience with 173 patients with cirrhosis upon whom 122 splenorenal and 51 portacaval shunts were performed, with an operative mortality of 12 per cent Linton still prefers the splenorenal shunt because he believes immediate and late mortality of this shunt is lower and postoperative neuronutritional difficulties fewer. He agrees, however, that splenorenal shunts carry a higher incidence of recurrent hemorrhage than do portacavals In his own experience with the medical-surgical quandary of survival Linton finds that under medical therapy alone only 50 per cent of patients were alive at the end of one year and 20 per cent at five years, in his group of patients selected for portal decompression, 80 per cent were alive at one year and 50 per cent at five years. Linton continues to recommend emergency ligation of varices for the immediate treatment of acute variceal hemorrhage.

Voorhees (28) records his and Blakemore's extensive experience with portal decompression In addition to the usual observations on mortality and morbidity, Voorhees states that 90 per cent of their patients surviving operation are alive at the end of their first postoperative year, 60 per cent at the end of their second through sixth years, 30 per cent at the seventh year, and 20 per cent at the eighth. In this series of 318 patients, hemorrhage re-

curred in 15 per cent after portacaval and in 45 per cent after splenorenal shunts.

Ferguson (29) reports on 40 shunts in 37 patients at the University of Minnesota. He affirms that results and mortality depend primarily upon the degree of selectivity exercised, that a large shunt which remains open affords permanent protection from variceal hemorrhage, that the end-to-side shunt is preferred, that shunts should not be performed for patients who have not bled, and that shunts are usually effective in the control of ascites. Splenectomy is unnecessary for hypersplenism attributable to portal hypertension provided a large portacaval shunt can be achieved Ferguson also records that six out of 10 of his patients bleeding from their varices but not decompressed, died of hemorrhage relatively promptly while four were alive one to six years later One out of six of his patients with varices but without bleeding ultimately died of hemorrhage while five lived four months to eight years with their varices but without hemorrhage. Ferguson, too, emphasizes that shunts may contribute to ammonia intoxication but in his experience this has been easily controlled by limiting dietary protein and administering neomycin orally.

Patton & Johnston's discussion (30) of the surgical treatment of portal hypertension summarizes the current dilemma about the precise etiology of portal hypertension and cause of variceal hemorrhage. They also discuss differential diagnosis, consider the urgent treatment of actively bleeding varices, and conclude that at present the side-to-side shunt is superior to the end-to-side In a subsequent technical article Large & Johnston (31) again emphasize their preference for the side-to-side shunt and plead that thoughtful surgeons consider wider application of this variety of decompressive technique.

Hsi-Ch'un's (32) article on portal decompression considers a large number of patients with schistosomal cirrhosis. Of 124 patients, varices which bled were present in 78 per cent, and 30 per cent had ascites. Ninety-five splenorenal shunts, 27 portacaval, and two lesser shunts were performed with a mortality of 9 7 percent Ammonia intoxication did not follow any of the splenorenal shunts but appeared in 11 of the 27 patients with portacaval shunts. Postoperative bleeding recurred in 5 per cent; in the remainder, the varices completely disappeared and bleeding did not recur Several patients were followed with regard to actual portal pressure for some days after operation. For instance, if preoperative pressure was 50 cm of saline, this fell to about 32 cm after opening the shunt, to 14, and then to 9 on the first and second postoperative days, respectively Contrary to most reports these investigators state that "splenorenal shunt lowers portal pressure more than portacaval does" This unique feature may bear some relationship to the high incidence (50 per cent plus) of schistosomal disease or to the enormous spleens carried by the patients in their series

Marion (33), Panke *et al.* (34), Welch & Ramos (35), Partington (36), and Sedgwick & Hume (37) have each reviewed their own series of patients

with portal decompression. These articles generally support the experiences listed above. Particular points of interest have been made by the following: Evans & Payne (38) report excellent correlation between roentgenographic subsidence of varices and relief from recurrent hemorrhage after adequate decompression by end-to-side portacaval shunts. Mikkelsen & Pattison (39) and Clauss *et al.* (40) write convincingly in support of urgent portal decompression for acute variceal hemorrhage. The latter accept hypothermia as a valuable adjunct to operation.

In most of the foregoing articles the splenorenal and end-to-side portacaval shunts have proved to be most popular. Two groups of investigators, Longmire *et al.* (41) and Large & Johnston (31) have each made strong pleas for the use of the side-to-side shunt. In the final analysis, both of these authors advocate this variety of shunt because it preserves an open pathway between the liver and the splanchnic bed Large & Johnston (31) believe that some portal blood must traverse the liver while Longmire (41) attempts to prove this by elegant experiments involving isotope tracers. There has yet to appear unequivocal proof that this hoped for hemodynamic relationship obtains to any appreciable extent in this variety of shunt—and that if it did it has yet to be proved that this is the critical factor in preventing post-shunt ammonia intoxication.

Early in 1958 Nachlas (42) published a polemic on venous shunts in the treatment of cirrhotic patients with esophageal varices He concluded, as have others before him, that a series of similar patients alternated between medical and surgical therapy is needed before final conclusions are warranted on the overall effectiveness of portal decompression. He even believes that there is abundant evidence to raise doubt about the value of shunts and hopes that his literary "effort will be considered worthwhile if it prevents patients from being subjected to a procedure of great risk and questionable benefit."

In May, 1959, two important articles (43, 44) appeared on surgical treatment of portal hypertension emphasizing that survival amongst patients selected for portal decompression must not be compared to survival of unselected patients representing the natural life history of cirrhosis. The oft repeated jingles of "70 per cent dead within a year" or "half die within two months" are invalid when dealing objectively with the long-term achievements of portal decompression. Taylor (43) writes that "Those who cite operative-shunt mortality figures must compare them with the flat survival curve as indicated after the first two to three months" and concludes once again that "patients upon whom portacaval shunts have been performed are a highly selected group, by virtue of having survived the ravages of the first or second massive hemorrhage." Hallenbeck (44) searched the records of the Mayo Clinic hoping to find patients with cirrhosis who had vomited blood but who had not been subjected to portal decompression. He expected to compare these with a similar group operated upon He failed in this intention because accurate data on liver function were not available among the

older patients in his series and because most of the patients he could find had undergone splenectomy often associated with omentopexy. From a slightly different point of view, his conclusions differ little from Taylor's. Hallenbeck writes "the principal reason for better survival in the surgical groups (portal decompression) is that the method used to select patients for operation also select those most likely to survive the longest."

Portal Hypertension in Children

The problems of infants, children, and young adults with hemorrhage caused by portal hypertension secondary to portal thrombosis, have been considered by Leger *et al.* (45), King & Shumacker (46), and Clatworthy & Boles (47) Each has emphasized the unpredictability of the extent of thrombosis, the proclivity of splenorenal shunts to close, and the rarity of success with caval-superior mesenteric shunts. Clatworthy (47) has summarized his experience with 11 infants and children He believes that extrahepatic block may early manifest itself by ascites and growth retardation. Later, ascites disappears, normal growth is resumed but variceal hemorrhages and hypersplenism may then appear. He concluded from his experience that the child under four years should not be operated upon, other than transesophageal ligation of varices in extenuating circumstances of uncontrollable bleeding. At about the age of four or even older a splenorenal shunt or a caval superior shunt should be tried Gibson & Rodgers (48) have reported portal hypertension as a complication of fibrocystic disease of the pancreas.

Esophagogastric Resection

When all efforts at portal decompression have failed, Habif (49), supporting Merendino & Dillard's (50) original efforts, supplies evidence that almost total gastrectomy and partial esophagectomy with jejunal interposition is effective in preventing further variceal hemorrhage. Deloyers *et al.* (51) have suggested partial esophagogastrectomy with esophagogastrostomy. The ultimate achievements of esophagogastric resection for varices have yet to be established but this form of treatment must certainly be considered when bleeding continues and all hope of successful decompression has been lost.

Ascites

When portal decompression was first practiced for esophageal hemorrhage, patients with ascites were widely accepted for operation in the hope that ascites too would be controlled. Mortality was high and soon surgical efforts in behalf of patients with ascites were abandoned Recently a number of clinics have returned to the enticing concept that ascites, refractory to

(56, 57) has reported complete relief of ascites following simultaneous hepatic and portal decompression (Fig. 1). Originally, ascites was believed to

subside after decompression because the shunt relieved the liver of the burden of splanchnic blood flow. Recently, however, critical attention has been given hyperaldosteronism in cirrhosis and ascites and its subsidence after portal decompression.

There is little doubt but that urinary output of aldosterone is excessive in patients with cirrhosis and ascites (58, 59). The relative importance, however, of apparent hyperaldosteronism, excessive antidiuretic hormone, hypoproteinemia, and portal hypertension is not yet clear if one judges from contemporary reports. Why urinary aldosterone should be elevated in pa-

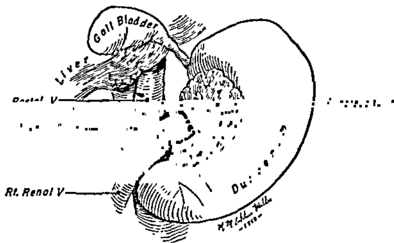


FIG 1 Diagrammatic Representation of the Operative Procedure Described as a "Double" Portacaval Shunt, Which Accomplishes Both Portal and Hepatic Decompression. (From McDermott, W V, Jr, *New England Journal of Medicine,* Vol 259, 1958)

tients with cirrhosis and ascites is unknown Excess secretion and failure of conjugation have both been suggested Henley (60) suggests that, since plasma volume is elevated in cirrhosis and since the major area of this increase is in the splanchnic bed and collateral channels, there might exist a deficit in systemic blood volume This in itself might promote excess secretion of aldosterone Wantz (61) and Luke (62) have each reviewed this subject; the former with greater emphasis upon the role of aldosterone and portal decompression; the latter reflecting the older thoughts with regard to levels of serum albumin, antidiuretic hormones, and medical therapy by diets low in sodium, ion exchange resins, and diuretics

Recently new steroids antagonistic to aldosterone have been developed (63, 64) Henley and his associates (60) report on the use of synthetic spirolactones SC 8109 and SC 9420 in patients with cirrhosis and ascites. Three of their patients experienced natriuresis and diuresis during the administra-

tion of these substances together with chlorothiazide. In these patients it was found necessary to follow serum potassium closely for spirolactones alone raised the serum level of this ion while spirolactone together with chlorothiazide depressed it. These investigators found themselves unable to explain fully the apparent hyperaldosteronism in patients with cirrhosis and ascites Experience with these substances has also been reported by Kerr *et al.* (65) working in Sherlock's department. SC 8109 was administered to three patients with cirrhosis and intractable ascites. Sodium and water diuresis occurred in two The third did not respond

Miscellaneous observations.—In addition to the observations reported above, less well categorized but important publications have appeared during the past year or so on problems tangential to portal hypertension With the development of a variety of tubes and balloons for exerting esophagogastric tamponade a number of casual references to the hazards of these devices have appeared Conn (66) studied critically the use of tamponade and concluded that it was associated with a greater risk than commonly believed. Of 50 patients subjected to tamponade, nine died as a direct consequence of its use while nine more had lesser but non-fatal difficulties. Only 20 were wholly free of complications.

Currently discussed is the possibility of an increased incidence of peptic ulceration after portal decompression. There seems little doubt but that gastroduodenal ulceration is more frequent in patients with cirrhosis than in the general population [Fainer & Halsted (67); Koide *et al.* (68)] but despite evidence of increased acid in dogs [Dubuque *et al.* (69)] with Eck fistulae it is not known how important peptic ulcer is as a complication of portal decompression in man. This subject is considered extensively by Clarke *et al.* (70), Silen & Eiseman (71), Clarke *et al* (72, 73), and Rousselot (74).

Moreno (75), working with Rousselot, has successfully shown that the severer the hepatic disease the higher the portal pressure. Gliedman *et al.* (76) have clearly demonstrated substantial falls in pressure following portal decompression Calvert *et al* (77) established, by two nicely documented cases, the syndrome of scleroderma and portal hypertension with varices and hemorrhage. Taylor, Jackson & Jensen (78) have shown that portal hypertension in Wilson's disease resembles that of extrahepatic block rather than that encountered in cirrhosis. Warren & Muller (79) have convinced themselves that portal hypertension is attributable to outflow obstruction and that the side-to-side shunt is superior to the end-to-side. Tisdale *et al.* (80) report five portal hypertension patients who were without demonstrable intrahepatic or extrahepatic obstruction and suggest "increased blood flow through the portal vein" as the cause of the elevated portal pressure in their patients.

Animal experiments.—Justice, in this review, can hardly be done to all of the studies in animals relating to the portal circulation. Most important are those of Laufman (81) who has succeeded in producing varices in monkeys by simultaneously occluding the inferior thoracic vena cava by 50 per cent

and dividing the azygos vein. Sixty to ninety days later the portal vein is ligated. In this primate, transient portal hypertension has been produced and large varices develop on the abdominal wall and in the esophagus. Portal hypertension, however, usually disappears within 90 to 180 days of portal venous occlusion but the varices remain. With such an animal preparation as this, the cause of variceal bleeding may soon be found. Davis *et al.* (82) have demonstrated in dogs that following thoracic caval constriction and the production of ascites aldosterone secretion rises promptly and reaches levels three to four times that of normal These experiments, together with other studies (83, 84, 85) of Davis and his associates, establish the important relationship of hyperaldosteronism to this variety of experimental ascites.

The liver of the dog has again been successfully arterialized by Cobb ✓ (86), employing first a reverse Eck fistula and later an arteriovenous fistula between the aorta and the vena cava. Owsley and his associates (87) have succeeded in demonstrating that portacaval transposition protects dogs from ammonia intoxication in experimental canine carbon tetrachloride cirrhosis Child *et al* (88) have succeeded in completely reversing venous flow through the liver in dogs Allen *et al.* (89) have produced ascites and modest portal hypertension in dogs by gradual occlusion of the hepatic veins alone. Grindlay (90) has confirmed again Pavlov's original observation that normal dogs with Eck fistulae die of meat intoxication. When, however, an Eck fistula is performed in dogs with experimental cirrhosis or with chronic portal obstruction, meat intoxication does not appear This, of course, may partially answer the question why humans with cirrhosis tolerate portal decompression as well as they do Grindlay admits these experiments throw "some mud in the water just to make this subject even more confusing."

Clearly, portal hypertension is a subject of continuing, vital, and even emotional interest to a wide variety of investigators in the clinic and in the laboratory. At the moment, each suggested solution to one or another of the many problems involved seems to raise a whole new set of questions to be answered. It seems safe to predict at this time that all of the obscurities of intrahepatic and extrahepatic circulation, both normal and abnormal, will not be resolved for some years to come

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HEPATIC COMA[1,2]

By Sheila Sherlock, M.D., F.R.C.P.
Department of Medicine, Royal Free Hospital and Medical School
University of London, England

The tempo of research into the problem of hepatic coma enjoyed an acceleration in 1954 with the observation by Davidson and his associates (1) that nitrogenous substances in the intestine might be injurious to patients with liver disease. The role of ammonia toxicity, described in the last century by the Pavlov school (2), was reinvestigated and further work proceeded on the general changes in nitrogen metabolism in patients with liver disease. The importance of the portal collateral circulation as a route from intestines to brain was emphasised particularly by McDermott & Adams (3) and by Sherlock and her co-workers (4). The last year has seen a slowing in the progress made and, although many publications are available for discussion, none provides a decisive breakthrough in eliciting the exact mechanisms of this serious condition. Many investigators still seem to regard hepatic coma as synonymous with ammonia toxicity and disregard other factors; the majority of contributions, therefore, deal with ammonia metabolism

General reviews of the pathogenesis and management have been published by Grossman (5), Sherlock (6) and, with particular reference to surgical patients, by Najarian and co-workers (7). De Groote (8) has written a thesis in Flemish on hepatic coma which has been sustained by the University of Louvain, Belgium; this includes a very full bibliography. The problem, with special reference to ammonia toxicity, has been discussed by McDermott (9).

Clinical Features and Diagnosis

The disturbance of consciousness, tremor, and hyper-reflexia of impending hepatic coma are sufficiently well known not to need repetition. The importance of adopting a clear clinical grading must be emphasized as well as the need for repeating the clinical assessment at frequent intervals. This is stressed by Summerskill (10) in an analysis published in French of a hundred cases of hepatic coma personally studied.

Davidson & Solomons (11) discussed the differentiation of impending hepatic coma from delirium tremens, a difficult problem in the alcoholic patient. In contrast to the patient with impending hepatic coma, the sufferer from delirium tremens is more alert, has more psychomotor activity and

[1] The survey of the literature pertaining to this review was concluded in July, 1959

[2] The following abbreviations will be used: EEG (electroencephalogram); CSF (cerebrospinal fluid); 5-HT (5-hydroxytryptamine); 5-HTP (5-hydroxytryptophan); HIAA (5-hydroxyindoleacetic acid).

anxiety, a finer tremor, more rapid speech, formed hallucinations, and autonomic imbalance.

The picture of hepatic coma can be difficult to distinguish from that arising from various electrolyte disturbances, some of which may co-exist with hepatic cirrhosis and even with true hepatic coma. Martini & Rausch-Strooman (12) describe a low-salt syndrome with hypochloremia, hyper-kalemia, and azotemia in cirrhotic patients given a rigid low-sodium diet and diuretics with or without abdominal paracentesis Weakness, lethargy, muscle spasms, and gastrointestinal distension were associated with oliguria. The condition was usually terminal, but temporary relief was sometimes produced by sodium chloride therapy. Hubble & Morris (13) describe potassium deficiency attributed to renal loss in two patients with cirrhosis The picture of muscle weakness may simulate hepatic coma Potassium deficiency can, of course, precipitate hepatic coma and this will be discussed later. Magnesium deficiency is said to be associated with disorientation, twitching, and muscle tremor. The picture bears only a superficial resemblance to that of hepatic coma although, as the serum magnesium levels tend to be low in alcoholics with cirrhosis (14), the distinction may have to be made.

Electroencephalography—It is difficult to have an objective yardstick to match against the clinical grading of hepatic precoma and coma. Although the EEG slowing is non-specific, serial changes in a patient known to have liver disease can be used for diagnosis and for following the progress. The late W. B. Hudson (15) proposed a simple classification of the EEG based on increasing appearance of slow waves and disappearance of normal alpha rhythms. Laidlaw (16) described a more elaborate method in which the frequency pattern of the EEG is constructed by measuring the speed of rhythmic activity automatically and the percentage time at each frequency; the mean frequency is thus calculated. This proves to be a very sensitive method of following the changes in the individual patient

Ammonia Metabolism

Estimation—The new method of Seligson & Hirahara (17), using potassium carbonate and bicarbonate for alkalinisation, has been accepted as satisfactory for determining ammonia in the blood. The ammonia is formed at a slower rate than in the older Seligson procedure In an excellent review of the methods used for ammonia determinations, Jacquez and his co-workers (18) finally recommend the use of arterial blood taken in a heparinized syringe and then iced. The estimation should be performed within 2 hr. on plasma by the Seligson technique

The evolution of ammonia is greater in oxalated than in heparinized blood. In the Seligson procedure, ammonia in heparinized blood from cirrhotic subjects seems to be liberated more rapidly than in normals, suggesting that proteins are present which contain more labile amide groups (19).

Significance of blood ammonia estimations—A consistently high blood

ammonia level in a cirrhotic patient is a bad prognostic sign, whereas a normal value gives no indication of the outlook (20). In a paper from China (in English) a good correlation was found between blood ammonia levels and the state of the liver and the portal collateral circulation (21). The arterial and jugular bulb ammonia content correlated with the clinical state better than did the venous level, according to Webster & Gabuzda (22). McDermott (23) found that blood ammonia determinations seemed to be of some value in the diagnosis of upper gastrointestinal hemorrhage, high levels being present in patients with bleeding esophageal varices. Unfortunately, Stahl & Bockel (24) found that cirrhotic patients, whatever the cause of the bleeding, had high blood ammonia values and the estimation could not be used to distinguish bleeding varix from bleeding ulcer. The level remained high while the bleeding continued and rose again if it recurred. Belkin & Conn (25) found similar results and also recommended using the blood ammonia and bromsulphalein tests together. A blood ammonia level exceeding 150 µg. per 100 ml with a bromsulphalein retention of 15 per cent was diagnostic of cirrhosis

The source of the blood ammonia.—The blood ammonia level represents a balance between ammonia production from the intestinal contents, removal of ammonia by the liver, ammonia uptake and release from peripheral tissues and brain, and ammonia production by the kidney. The part played by these organs varies from time to time, and in particular depends on whether or not the patient has liver disease or whether he is in hepatic coma.

The intestinal ammonia production largely depends on the protein content of the intestines and their bacterial activity. Lawrence and his coworkers (26) have shown in dogs that blood ammonia rises after hepatectomy but this is prevented by evisceration. This again emphasises the importance of intestinal flora in ammonia production. It is of interest, however, that infants, during the first few hours of life, frequently show raised (1 µg. per ml) blood ammonia levels (27) This is in spite of absent putrefactive activity in the intestines, which are sterile at birth. The liver finally converts the ammonia to urea Pearl & McDermott (28) found raised plasma citrulline levels in patients with disorders of the liver and portal circulation, and believed that its conversion to arginine (Krebs-Henseleit ornithine cycle) had slowed and that this was a rate-limiting and vulnerable step in urea synthesis in man

In normal subjects an increased peripheral uptake of ammonia follows intravenous infusion (29). Artz and his colleagues (30) observed in dogs that ammonium acetate infusions resulted in uptake by peripheral tissues and brain while the infusion was running and a reversal when the infusion stopped. Ammonia diffused freely into the cerebrospinal fluid. Webster & Gabuzda (31) noted a decreased uptake of ammonia by the peripheral tissues and brain in hepatic coma In some instances, the brain might even release ammonia

Dawson *et al* (32) found that the increased arterial ammonia concen-

tration after acetazolamide (Diamox) could be related to diminished peripheral uptake, and the fall in blood levels after the amine oxidase inhibitor, iproniazid, could be related to increased removal at the periphery (33).

Mechanisms of Cerebral Toxicity

The biochemical aspects of cerebral dysfunction have been well-reviewed by Quastel & Scholefield (34). Theoretically, Bessman (35) postulated that ammonia intoxication could interfere with cerebral metabolism by glutamine synthesis and by reductive amination of α-ketoglutarate. The ketoglutarate would be removed from the Krebs cycle and so diminish the formation of other members of the cycle with reduction of oxidative phosphorylation in the brain. Much work has been done to prove and disprove this theory. Using ^{15}N-labelled compounds in rats, Duda & Handler (36) have shown that glutamine synthesis is the major fate of ammonia. Gilon *et al.* (37) have observed glutamine levels of 23 to 96 mg. per 100 ml. in the CSF from patients with hepatic coma. In liver disease without coma, or with coma resulting from other causes, the levels never exceeded 35 mg. per 100 ml. A rise in the glutamine content of the CSF is believed to be pathognomonic of hepatic coma. Clark & Eiseman (38) analysed brain tissue from dogs rendered comatose by infusion of ammonium salts into the carotid artery and found a fall in the concentration of α-ketoglutarate and a rise in pyruvate and glutamine. Ulshafer (39) found a lower acetylcholine content of the brain in rats following ammonium ion intoxication. This could be caused by the ammonium ion converting glutamic acid to glutamine and so depriving the brain of acetylcholine at the synapses since glutamic acid is essential for the formation of acetylcholine. A "biochemical decerebration" is thus produced. These results support the Bessman hypothesis. In contrast, Handford (40) produced reversible ammonia intoxication in normal dogs by intravenous ammonium sulfate or ammonium carbonate. The blood ammonia and urea rose but there was no significant change in the plasma α-ketoglutaric acid or glutamine levels. It has been repeatedly shown that the blood α-ketoglutaric acid level rises rather than falls in hepatic coma and that this is in proportion to the severity (41, 42). Walshe and his co-workers (43), using slices of rat brain cortex, found that ammonia was a normal brain metabolite and that there was no clear evidence to indicate that glutamine synthesis was a rapid or efficient method of its removal. In a very stimulating discussion, Walshe suggests that the raised blood ammonia reported in hepatic coma may *well be more of a non-specific indicator of disturbed brain metabolism than the toxic factor which* causes this. There is, at present, no evidence to show that ammonia is toxic to the normal brain at concentrations which have been reported in man. Two factors appear to be involv ... the first is a deficiency of enzymes or e ... phosphate bonds in the brain, an normal (or marked excess of a r

Respiratory alkalosis is a common accompaniment of hepatic coma and carbon dioxide inhalations have even been suggested in treatment (44). Lawrence *et al.* (45) have shown in dogs that alkali increases total ammonia production and free ammonia shifts into the tissues Stauffer & Scribner (46) observed tremor, apathy, and confusion with EEG changes in a patient with severe alkalosis but with normal liver function given intravenous ammonium chloride, and suggested that alkalosis might augment ammonia toxicity. These observations have been confirmed by animal experiments. Warren (47) noted that the toxicity of ammonium salts in mice was related to their effectiveness in raising the blood pH. The change seemed to be associated with an effect of pH on the NH_3-NH_4^+ ratio and the ability of ammonia gas to cross the blood-brain barrier, or to a direct effect of pH on the barrier. Further work showed that in mice the rate of passage of ammonium salts across the blood-brain barrier was indeed related to the different effects on blood pH (48). As the blood pH rose the unionised ammonia increased relative to ionised, and so crossed the blood-brain barrier. It was then noted in dogs that, in the steady-state, brain, muscle, and CSF ammonia values correlated with the arterial ammonia level. After the infusion of acid or alkali, the diffusion of ammonia into the CSF was related to the direction of pH change between blood and CSF. There was a direct and predictable correlation between alterations of blood pH and the tissue ammonia content (49). The hypothesis was finally tested in the human subject with impending hepatic coma by Warren and his colleagues (50). Following infusions of acid or alkali, the arterial pH changed but the CSF did not, resulting in an altered pH gradient between arterial blood and CSF. This change in the pH gradient was associated with a predictable alteration in arterial-CSF ammonia ratio In a limited number of observations on patients with hepatic coma, in spite of an increase in the arterial-CSF ammonia ratio following acid infusions, clinical benefit did not ensue.

The deleterious effect of alkalosis in patients with liver disease might be correlated with the observation that certain diuretics, especially chlorothiazide, are liable to be followed by hepatic come (51 to 54). Sherlock *et al* (51) noted that chlorothiazide was often followed by precoma in patients with cirrhosis and, extending that work, Read and others (52) reported that chlorothiazide, 2 gm daily, induced impending or actual hepatic coma in 7 of 13 cirrhotic patients. It was usually associated with a good diuretic response to chlorothiazide and a previous history of hepatic precoma. The coma was related to a large potassium diuresis and Read and his associates (55) further noted that potassium supplements resulted in clinical improvement and return of the EEG toward normal even though chlorothiazide was continued Similar changes could be induced in three patients with cirrhosis by potassium depletion produced by diet and exchange resins. In every instance, urinary potassium rose, serum potassium levels fell, and serum alkali reserve and blood pH rose Patients showing neuropsychiatric changes had a rise in arterial blood ammonia values. The development of precoma

was believed to be *related to the potassium deficiency and its accompanying* extracellular alkalosis Mackie and his co-workers (54) could prevent the development of neurological complications after chlorothiazide by giving broad-spectrum antibiotics but this was not so in one patient who was studied by Read *et al.* (55). Kerr and his associates (56) *have noted that* hydrochlorothiazide can also induce hepatic precoma in cirrhotic patients.

Amines absorbed from the intestine might be more toxic to the brain than ammonia. Asatoor & Dalgliesh (57 [illegible] blood amine levels of 13 normal su[illegible] compared with 26 patients with liv[illegible] ml.). In one patient *iso*-amylamine and β-phenylethylamine were detected in blood.

Serotonin or 5-hydroxytryptamine (5-HT) may play an important part in cerebral metabolism and a low normal excretion of 5-hydroxyindoleacetic acid (5-HIAA), the end product of serotonin metabolism, has been reported in liver disease (58). Borges *et al.* (59) have therefore investigated the possibility that severe liver disease might affect the production of 5-hydroxytryptophan (5-HTP), the apparent precursor of serotonin in brain. 5-Hydroxytryptophan (10 to 40 mg) was therefore given intravenously to nine patients and EEG's were recorded Five patients with hepatic coma showed diminution of slow activity and increase of fast activity although *there was no clinical change. Three with coma not caused by liver disease,* and one normal subject showed no change in the EEG No definite defect was observed in the ability of patients with liver disease to metabolise 5-HTP compared with normal. The urinary excretion of 5-HIAA was in the low normal range Donaldson and his co-workers (60) also found 5-HIAA excretion the same in cirrhotic as in normal subjects whether or not coma was present. After 5-HTP infusions the excretion of 5-HIAA was greater in cirrhotic subjects, and all six patients with greater than 70 per cent excretion were showing encephalopathy. A failure of conjugation of 5-HT in cirrhosis was postulated

Precipitating Factors

Blood in the intestine is perhaps the commonest precipitating factor causing hepatic coma in cirrhotics This combines a protein load, depression [illegible]

[illegible] *protein hydrolysate solution led to impending* hepatic coma and increased venous ammonia concentration in two patients described by Webster & Davidson (62). The solutions sometimes contained ammonia but degeneration of infused amino acids provided a further source of ammonia

Silberman (63) described hepatic coma developing in a cirrhotic patient following ureterosigmoidostomy The urea in the intestine was presumably the source of ammonia. Similarly, Webster & Gabuzda (64) reported coma

in four patients with cirrhosis becoming azotemic and they believed that circulating urea may lead to high blood ammonia levels. It was also noted that intravenous urea was followed by rises in the ammonia level in a portal collateral vein and that this could be prevented by neomycin (65).

Chronic neurological complications can be expected in many patients after portacaval anastomosis (3, 4, 66). The immediate rise in arterial ammonia concentration after operation seems to arise from liver dysfunction (67) but continued increases are presumably related to the shunt.

The precipitation of hepatic coma by diuretics has already been discussed.

TREATMENT

General reviews on the management of hepatic coma have been published by McDermott (68), Manning & Delp (69), and Najarian & Harper (70).

Dietary protein restriction and antibiotics—It is now generally agreed that patients in hepatic coma should be given little, if any, protein by mouth. Webster & Davidson (65) noted increases in ammonia content in abdominal portal collateral vein after oral protein.

The toxic factors interfering with cerebral metabolism seem to be produced by bacterial action on protein in the intestine It is therefore of value to diminish bacterial action in the gastrointestinal tract. Chlortetracycline may be effective for short periods but resistant organisms soon emerge (71) and staphylococcal enterocolitis is a real risk. Faloon & Fisher (72) treated 22 patients with neomycin, 11 survived and 9 others improved prior to death. A dose of 4 gm daily seemed too little and 12 gm daily too much, so that 8 gm. daily may be an appropriate amount. No significant difference in the results was noted between those given more or less than 25 gm. of protein daily. Stormont and his co-workers (73) noted that some protein could be given after two days provided the patient received neomycin or paromomycin Summerskill (74) found neomycin of more value than chlortetracycline or chloramphenicol in hepatic coma following gastrointestinal hemorrhage, and it permitted an adequate protein intake. He found that in patients with considerable severe, irreversible, liver disease the maintenance of a clear mentality might be undesirable · " the distressing and unequal struggle seems to be unduly prolonged"

Oral neomycin, when continued for long periods, may be absorbed and Last & Sherlock (75) found detectable blood levels on 11 occasions in 7 patients treated for hepatic coma. One patient suffered permanent deafness. Renal toxicity could not be incriminated

Investigating other broad-spectrum, poorly absorbed antibiotics Faloon & Fisher (76) found that kanamycin (8 to 12 gm. daily) reduced blood ammonia levels, but with continued use the values fluctuated so that neomycin was probably the superior antibiotic for hepatic coma. Fast *et al* (77) believed that paromomycin was as effective as neomycin used in a dose of 4 gm. daily.

Glutamic acid and arginine—These substances are recommended for

combating ammonia toxicity. Glutamic acid (given as monosodium glutamate) might combine with ammonia to form innocuous glutamine, and L-arginine might increase the production of urea from ammonia by stimulating the Krebs ornithine citrulline arginine ... cycle. Aft

encephalopathy but even in this group Fazekas and his associates (78) report only temporary depression of blood ammonia levels and no change in clinical state, cerebral oxygen consumption, or EEG Wewalka (79) recommended giving 200 to 500 ml. 10 per-cent sodium glutamate intraperitoneally, and 10 of 26 patients with hepatic coma so treated were able to leave hospital.

Najarian and his co-workers (80) treated 90 patients with L-arginine and noted that a reduction of blood ammonia was usually followed by improvement in the clinical status. The overall mortality was 32 per cent. The clinical state correlated better with the CSF ammonia level than with the venous value. Fahey found that L-arginine protected against the toxic effects of amino acid infusions in cancer patients (81). Later, he and his co-workers (82) found that arginine, in dogs, given with glycine caused the liver to take up ammonia. However, L-arginine had no effect on ammonia already

pathy (83). Neither did it affect blood ammonia elevation by exogenous administration of ammonium salts These workers conclude (83) that

Reynolds *et al.* (84) found that marked clinical improvement followed arginine administration in 2 of 34 instances, and followed a placebo in 1 of 26 instances The mean arterial ammonia level did not change significantly in the treated group compared with the control. A beneficial effect from L-arginine could not be demonstrated Wolfe *et al* (85) have used arginine and DL-ornithine (a further Krebs urea cycle intermediate) and conclude that these and other agents solely designed to reduce ammonia concentration by intravenous infusion have limited value in the treatment of hepatic coma

Hemodialysis.—Kiley and his co-workers (86) have treated patients with cirrhosis and gastrointestinal hemorrhage by a Kolff-type artificial kidney. In one patient, 41 litres of blood was dialysed in 4 hr. and 71 mg NH_4N was removed, the blood ammonia level fell from 280 to 180 µg per 100 ml Four patients showed clinical improvement after effective dialysis Hemorrhage had always ceased and the necessary heparinization did not prove to be a problem. Schechter and his associates (87) used a specially prepared cation exchange resin in the sodium cycle to reduce blood ammonium levels in dogs. One patient in hepatic coma similarly treated showed a fall in arterial ammonia level and great improvement. Replacement of calcium,

potassium, and magnesium may be necessary. Such procedures are clearly in the experimental stage, and more work is needed before their place in therapy can be evaluated They will prove to be of particular value in patients going into coma after a sudden nitrogen load such as gastrointestinal hemorrhage. Even more interesting, but still, of course, in a preliminary stage, is the use of a donor liver. This removed ammonia from the blood of an Eck fistula dog and also produced bile and extracted bromsulphalein (88).

Corticosteroids.—Webster & Davidson (89) treated seven cirrhotics in spontaneous impending coma or coma with large doses of cortisone or hydrocortisone. Four showed temporary improvement but all eventually died in hepatic coma These compounds did not reduce the mortality in patients with hepatic coma but might prove of permanent benefit given early to patients who have sufficient hepatic reserve to survive after temporary improvement. Pessar & Hessing (90) treated six patients with hepatic coma and cirrhosis with large doses of cortisone With the exception of one case treated inadequately, all the patients came out of coma dramatically and quickly. However, all eventually died of various complications including coma.

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CARDIOVASCULAR DISEASE: ARTERIAL HYPERTENSION[1,2]

By Maurice Sokolow, M.D. and Paul J. Sanazaro, M.D.[3]
Department of Medicine and Cardiovascular Research Institute, University of California School of Medicine San Francisco, California

The past five years have seen remarkable clinical advances in the field of HT: proof of effective drug control of severe HT and recognition of two major causes of "curable" HT—renal artery lesions and aldosteronism While numerous clinical, epidemiological, and experimental observations have illuminated various facets of the field, they have produced more speculations than direct answers to questions of etiology and pathogenesis. Restrictions of space limit this review to a brief survey of significant trends

Pickering's (1) book is a masterful presentation of the subject, emphasizing particularly the physiological and conceptual aspects. A study of 114 hypertensive patients relating personality to various physiologic parameters, including details of frequent interviews in 26 cases, is presented by Wolf and co-authors (2) The symposium of the Wellcome Foundation on Hypotensive Drugs (3) covers the pharmacologic aspects and clinical applications of hypotensive drugs and control of vascular tone Smirk's excellent book (4) emphasizes the pathogenesis of the disease and the specific details and effectiveness of therapeutic agents. A sound clinical discussion of HT disease by Hoobler (5) will be of considerable benefit to the practitioner Moyer (6) has edited a superb symposium of various aspects of HT. The sections dealing with the basic concepts of etiology and the details of therapy are particularly recommended. Many excellent physiologic papers are included in the Michigan Symposium (7) Clark, Glock and their associates (8) report on a five-year program of planning and research in the epidemiology of HT An early symposium on the management of essential HT (9) offers valuable background information A group of 53 papers presents some previously unavailable European work (10) Simonson & Brožek's (11) comprehensive review on Russian research on HT contains many references unknown to us, including a monograph, *Hypertensive Disease*, by Lang, published in 1950, which is said to be a basic reference book and guide for most of the work on arterial hypertension in Russia

[1] The survey of the literature pertaining to this review covers the period January, 1955, to July, 1959

[2] The following abbreviations will be used BP (blood pressure); DP (diastolic pressure); HT (hypertension or hypertensive, as it applies); MAO (monoamine oxidase activity); SP (systolic pressure)

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years, approximately half developed fixed HT (18). This occurred twice as often in patients with known family predisposition to HT.

The Pathogenesis of Primary Hypertension

Age, heredity, and psychological factors undoubtedly play a role, but the relative contribution of genetic and environmental factors is unknown.

Age—Perera (19) states that although the average, casually recorded BP of a population sample tends to rise with age, the resultant curve is made up of multiple components: primary HT, secondary HT, atherosclerosis of the aorta, and transient rises from physiological causes, obesity, or the emotions.

Almost all population studies (1, 20, 21, 22) show a rise in BP with age, but whether aortic atherosclerosis alone is responsible is not known. Clarification requires longitudinal studies in patients who do not develop atherosclerosis of the aorta Padmavati & Gupta (23) noted that malnourished Indians neither gain weight nor show a rise in BP as they grow older, whereas Indians with a higher standard of living show increases in both weight and BP with increasing age The fact that the rise with age is mainly systolic supports the idea that it results from changes in the aorta and great vessels, rather than in arterioles Data from Master *et al.* (24) and Anderson & Cowan (25) suggest that DPs greater than 112 mm. Hg are probably abnormal

Veale *et al.* (26) found that although the near basal BP (obtained by multiple readings on a second occasion) rises slightly with age, there is roughly 30 to 40 mm difference in pressure between the first casual and the near basal at all ages. The closer one approached basal readings, the less was the rise in pressure with age.

Pickering's data (1) reveal that DP rise is greater between the ages of 30 and 50 than it is between the ages of 50 and 70, although SP was considerably greater in older age groups Furthermore, the variability of BP also increases with age

Inheritance—Pickering (1) concludes that the BP level is inherited as a graded characteristic of the whole range of pressures ordinarily encountered Environmental factors may then modify the inherited level and be of greater moment than the genetic factors Miall & Oldham (20, 27) in their definitive population studies found no evidence of bimodality; they found similar regressions for arm girth scores and SP scores, and suggested that the inheritance of arterial pressure may be entirely explicable in terms of arm girth or weight The difficulties of population surveys and the need for samples representative of the general population are well discussed

Thomas (28) found a threefold greater incidence of HT in siblings of HT patients than in siblings of normotensive individuals Precursor traits, such as overweight and higher and more labile BP and heart rates, were also more frequent in students with a parental history of HT The indi-

Concept of Primary Hypertension

Page and his associates (12) discuss the *mechanisms of HT*, *concluding* with their "mosaic" theory which incorporates the wide variety of normal and pathologic mechanisms that influence BP Lang, according to Simonson & Brožek (11), believes that HT results from prolonged psychic stress which lessens normal cortical inhibition of the hypothalamic vasomotor center, *thereby resulting in abnormal BP rises which are initially transient* Renal, endocrine, cardiac, and cerebral mechanisms are thought to be important in the later phases. Lang's emphasis on the role of psychic trauma is the most forthright of any current group of workers

Pickering (13), in presenting his concept of essential HT, points out that *the difference between normal and abnormal BP is not so much a* natural division as an arbitrary statistical device. He also distinguishes between arterial disease and HT and concludes that from a prognostic standpoint vascular involvement is more important than the height of BP. The concept of "normal" BP is also discussed by Hines (14) and by Stewart (15). *Insurance authorities define an elevated pressure as one indicative* of increased risk (greater than 140/90 mm Hg) Pickering, although emphasizing the lack of a clear dividing line between normal and abnormal, does not deny the increased risk associated with pressure in the upper range of the distribution curve

The Natural History of Primary Hypertension

Perera (16) reports the natural history of 500 patients with HT treated solely with symptomatic measures Of particular interest is the unique group of 150 patients whose known average ages at onset and death were *32 and 52 years, respectively Average survival after the development of* cardiac hypertrophy was eight years and after various coronary or cerebal atherosclerotic accidents, four to five years Patients who developed arteriolar necrosis with papilledema survived only one year; an occasional patient lived 18 years after atherosclerotic complications The disease *progressed at highly variable rates, and death was more closely related to the* effects of cardiac hypertrophy and of arterial and arteriolar sclerosis than to the casual BP level. Evelyn (17) found that in 99 per cent of subjects with essential HT, the disease had appeared gradually, then passed through a transitional period of several years, during which rises in BP, at first intermittent, became more frequent and sustained *In a few exceptional* cases, the transition from a normotensive to an HT state was abrupt, and the disease progressed rapidly *The early phase of HT was asymptomatic*, and the development and relative degree of vascular complications varied widely, as in Perera's series The discrepancies between height of BP and rate at which complications developed, noted by both Evelyn and Perera, may stem from the prognostic unreliability of casual readings. Of a group of 345 patients with *transient* elevation of BP who were followed up to 20

HT, HT heart disease, and cardiovascular disease are more frequent in Negroes than in whites. Schroeder (38) finds that primary HT is relatively common in the Orient, the incidence ranging from 5 to 37 per cent.

Psychological factors.—Although stress is a plausible etiologic factor in essential HT, the evidence is primarily indirect (39, 40, 41). Most investigators agree that emotional stress aggravates existing essential HT and, in turn, the vascular complications, but how or to what degree it participates in the pathogenesis of the disease is not clear.

It is [illegible] triction in the k [illegible] potent drugs, s [illegible] account for the observed lability in BP and variations in the rate and progression of vascular complications. Psychiatric data on temporal relationships between significant life experiences and onset and exacerbation of HT suggest a close association but not necessarily one of cause and effect.

Direct experimental demonstration that recurrent psychological injury can produce chronic vasoconstriction and a clinical picture similar to HT is lacking except for a Russian report (42) that such trauma produced persistent HT in the monkey Since the etiological importance of long-continued psychological stress cannot be proved experimentally in man, the evidence must continue to be circumstantial. Shapiro & Horn (41), using the Masserman technique, produced chronic anxiety in cats but were unable to cause persistent HT in the conditioned animals. Shapiro & Melhado (43) produced experimental HT in 17 rats by a figure-of-eight tie on one kidney and contralateral nephrectomy, then exposed nine of the rats to psychological stress with a buzzer and electric shock All 17 developed HT and some impairment of renal function, but the latter did not correlate with BP levels. In the nine exposed to stress, the manifestations of the HT process were more severe

Aggravation and pathogenesis should be considered as separate, not identical, factors. It is well known that emotional stimuli induce rises in BP but, as Grollman (44) comments, the crucial point is whether repeated pressor stimuli induce chronic HT, not whether stresses stimulate the sympathetic nervous system, thus raising BP. Wakerlin (45) argues that if the stress mechanism induces HT by release of renal pressor substances then this must also occur in people who do not develop HT except on a temporary basis Wakerlin suggests that some basic latent biochemical lesion in the kidney, or in other organs, may initiate HT, stress being merely a trigger mechanism. Cohen and his associates (46) demonstrated marked variations in black-out levels in pilots in high-speed aircraft and subjects in a human centrifuge, which cannot be accounted for on a physiological basis and presumably are related to personality structure Goldberger (47), in a comprehensive discussion, concludes that psychological factors are involved in the etiology of HT, and presents a reasoned mechanism of production

vidual with precursor traits responded to stress and *to smoking with an* abnormally high cardiac output as measured by ballistocardiography. Hines (14), in a follow-up study of 20 years, found subsequent HT in 44 per cent of 265 cases with a positive family history, and in only 7 per cent of 442 with a negative family history. Kohlstaedt *et al* (30), in evaluating the genetic and environmental factors in HT, emphasize the need for longitudinal studies, such as the Framingham survey which demonstrated a reduced life expectancy even with mild degrees of HT (31). Grollman (32) reiterates his view that the most plausible explanation for the genesis of HT, in the light of current clinical and experimental information, is the presence of a congenital functional defect of the kidneys.

Obesity.—Whyte (33) concludes that increased body weight, but not obesity, influences the level of the BP. The relationship between weight and BP was minimized by Bøe and his associates (21), who surveyed the entire population of Bergen, Norway. Bjerkedal (34), in a study of 14,784 adults, also notes that HT was no more frequent in overweight patients than in those of average weight and underweight.

Racial and population studies—Schrire (29), in South Africa, reports that the incidence of HT is approximately 30 per cent in Bantus, "Cape colored," and Caucasians alike Myocardial infarctions were extremely rare in the Bantu, intermediate in the "Cape colored" and common in the European *The high incidence of HT in both the non-whites and whites in* South Africa effectively negates previously published data that HT is rare in the African Negro.

Fraser (35), in a study of 2000 Bantus and "Cape colored," found HT in 12 per cent. Of these, 26 per cent were primarily renal in origin; of 47 fatal cases, 21 or almost 50 per cent, had underlying renal disease Uys (36), however, reported a low incidence of nephrosclerosis and a normal incidence of glomerulonephritis in the Bantu at autopsy Fraser confirmed the rarity of coronary disease and lower incidence of heart failure in the Bantu than in the white South Africans, despite the comparable incidence of HT The low incidence of coronary disease in the Bantu, despite the relatively common HT, may be related to low serum cholesterol and low fat intake; HT, per se, may not be as important in accelerating coronary disease *in these patients as it is in Europeans who have both high pressures* and relatively high cholesterols (compared to the Bantu).

Salem (37), in Egypt, notes that 51 per cent of 1500 out-patients over the age of 40 had DPs of 100 mm Hg or more. Renal disease was found in 16 per cent of the HT patients, and of these, 8.5 per cent had malignant HT. In contrast, the studies of Padmavati & Gupta (23) on 1000 Indians indicate an incidence of HT of only 2 6 per cent in the rural farmer, and 1 2 per cent in the industrial workers of Delhi Coronary disease was practically nonexistent.

Clark *et al.* (8) summarized many papers which agree *that essential*

longed rise in BP. He also noted that adverse factors in the social surroundings were much more common in the HT than in the control group.

Shapiro & Melhado (55) exposed rats to various conditioning procedures designed to provoke chronic anxiety. Chronic organic disease did not develop in the animals after 12 weeks, but there was evidence that pre-existing HT could be aggravated.

Wakerlin, in a timely and lucid editorial (56), summarized his views regarding pathogenesis.

Prognosis of Hypertensive Vascular Disease

The introduction of potent ganglionic-blocking drugs has increased the need for a clearer understanding of the natural history of untreated primary HT if we are to assess accurately the results of such therapy.

Basal vs. casual blood pressure—Which is more important from a prognostic standpoint, the floor (basal) or the casual pressure? Several good studies indicate that basal BP is more important, but casual BP cannot be ignored. Perera (57), analyzing 50 patients with HT vascular disease whose total disease histories were known, found that the average age at death in patients with labile HT was 56 years, 23 years after the known onset of elevated BP, and in "non-labile" patients was 44 years, 13 years after known onset. The age at onset in the two groups was almost the same, but the total duration of HT differed significantly The average casual maximum BP was essentially the same in the two groups, but the range was wider in the labile patients

Smirk (4), long interested in basal pressures, presents data on basal and casual readings in 1000 patients; usually repeated readings were made during a special early morning visit. The incidences of cardiac hypertrophy, cardiac failure, and death rate which differed strikingly, are shown by Smirk to correlate with the basal readings, even in patients whose casual pressures are equivalent He emphasizes that the prognosis is worse when the basal reading is high. Further supporting evidence is presented by Simpson & Gilchrist (58), who reported that the five-year survival progressively decreased as resting DP exceeded 110 mm Hg and amobarbitol (Amytal) sleeping pressures exceeded 90 mm Hg; a casual DP greater than 140 denotes a poor prognosis, although five-year survival rates were the same between levels of 110 and 140 mm Hg. During a two-year period in Fraser's series (35), mortality was 62 per cent in patients with severe sustained HT but only 11 per cent in patients whose BP dropped to normal after admission to hospital. Mathisen *et al* (59) report a 15-year study of 290 patients whose mean age at registration was 37.5, none of whom had received specific hypotensive therapy. The death rate was about five times higher in patients with more stabilized HT than in the group with labile HT.

The Russian point of view, noted in Simonson & Brožek's review (11), emphasizes the importance of psychogenic disturbances in initiating HT. Mills (39) summarizes the physiological and metabolic responses to various types of stress and suggests ways in which changes in BP could be initiated and then maintained after the initiating factor is no longer operative. Bliss and his associates (48) have determined that the adrenal cortical response to the stress of life situations and of experimental conditions is less than the response to intravenous administration of ACTH, pyrogens, insulin, electric shock treatment, or moderate exercise. Connell *et al.* (49) found significant increases in the urinary excretion of adrenal cortical hormones after an important academic examination. Brod (50) noted that various unfavorable factors in professional and home life caused adverse changes in BP regulatory mechanisms He referred to Polish data suggesting that juvenile HT in most instances is equivalent to early HT disease, and can be "cured" at this early stage by a radical change in social environment. He feels this is one of the most important preventive steps to be taken and, although the paper does not state whether the elevated BP was transient or more persistent, this is one of the first definite attempts to treat the so-called pre-HT phases of the disease by change of social environment.

Moses and his associates (51), studying the physiologic responses to psychodynamic situations, concluded that the HT vascular process would be improbable in the absence of initiating psychic stresses operating through the autonomic nervous system They believe the resulting BP elevations may persist even though the psychic stresses subside, and also, on the basis of six documented remissions, that psychotherapeutic modification of early HT vascular process is possible. Significant correlation has been reported between the resting mean BP and the intensity of acute fear and anger responses in normal and HT subjects (52). Kalis and her associates (53), using the technique of psychodramas, found that HT women, compared to normals, had poorer emotional and behavioral control, were less flexible and adaptive in the stress situation and lacked appropriate assertiveness. HT subjects, as well as pre-HT women described in a previous study, apparently perceived stress more readily and handled it less well; they appeared to be more vulnerable to repetitive rises in BP which lead ultimately to essential HT. A four-year follow-up of pre-HT women and matched controls disclosed no basic change in personality characteristics that differentiated pre-HT women originally and which are found in adolescents with pressures in the higher range of normal and in HT patients (54). The consistent findings in these several groups indicate that abnormal psychological reactivity to stress antedates HT Although resting BPs were essentially the same in both groups, pre-HT females could be distinguished by the fact that breath holding resulted in DPs exceeding 90 mm. Hg. Brod (50) found that the majority of his HT subjects, in contrast to normal controls, reacted to the stimulus of mental effort by an exaggerated, pro-

Hypertension of Pregnancy

Finnerty (63) differentiates toxemia of pregnancy from HT vascular disease by the presence of a generalized retinal sheen in the former. Of 154 toxemia patients, 125 had no symptoms or signs (e g, elevated BP, edema, albuminuria, or retinal sheen), six weeks postpartum. Definite grade I and grade II retinopathies, however, were present in 29 patients whose BP continued to be normal. The literature with respect to the variability in diagnosis of toxemia is discussed in detail by Schreier *et al.* (64). Biopsy of renal tissue obtained from 72 pregnant women with toxemia and preeclampsia revealed moderate thickening of the basement membrane of the glomeruli and narrowing of the glomerular capillaries (65).

A reduction in the incidence of HT in pregnancy from 8 7 to 4 7 per cent in the decade between 1944 and 1954 is attributed to limitation of sodium intake (66). The use of oral and intraveous protoveratrine in over 109 patients with toxemia resulted in almost all cases in complete control of pressures within 24 hr. (67). Finnerty *et al.* (68) report on the remarkable effectiveness of chlorothiazide in the treatment of toxemia of pregnancy.

Hypertension in Infants, Children, and Adolescents

The frequency of HT in childhood and adolescence is unknown, and BP determinations are being urged as a routine measure in examining children and adolescents (69). A useful paper (70) has appeared on the technique of measurement of BP in infants and children and the importance of cuff size; normal values are tabulated. The differential diagnosis of HT in childhood and a number of cases of essential HT are reported (71 to 74). An excellent summary of the current status of treatment of HT in childhood with and without renal disease is presented by Daeschner & Dodge (75).

Pathology

The weight of evidence increasingly favors the conclusion that renal vascular changes in essential HT are secondary to HT and do not, as Goldblatt maintains (76), precede and cause HT. The most thorough paper on the associated pathological changes in the kidney and the adrenal gland in HT was recently presented by Sommers *et al.* (77) and Shamma *et al.* (78). He reviewed, without prior knowledge of the clinical data, material from 313 nephrectomy, 1973 renal biopsy, and 97 adrenalectomy specimens. He also compared pathologic changes of the adrenal in specimens taken at autopsy from 220 HT and 220 normotensive individuals. No renal vascular lesions were found in 1.6 per cent. A consistent relationship existed between the grade of arteriolar thickening and average DP. Pyelonephritis was present in 15 per cent of cases with significantly higher than average DPs and less favorable prognoses The adrenal glands were normal in 80 per cent of HT cases, but a true adrenal cortical adenoma was found in 20

These five papers support the concept that casual readings of arterial pressure are misleading prognostically, unless DP consistently exceeds 130 mm. Hg. Resting and basal levels correlate more closely with total duration of life and average age at death A lack of lability points toward a less favorable course, but labile HT is not a guarantee of continued health. Failure to consider basal pressures may explain the apparent discrepancies between observed improvement in vascular complications and lack of satisfactory BP response to various therapeutic procedures, such as sympathectomy and ganglionic-blocking drugs.

Vascular complications—Prognosis is adversely affected by the presence of vascular complications, notably cardiac hypertrophy or failure, HT *neuroretinopathy, renal impairment with albuminuria, or cerebral vascular* accidents (16, 17). Smirk (4) emphasizes that five-year survivals are considerably rarer in patients with vascular complications; therefore, treatment should be instituted when such complications appear. Hoobler (5) finds that only 20 per cent of patients presenting vascular complications, compared to 80 per cent without, survived 10 years. Simpson & Gilchrist (58) demonstrate close relationships between resting DPs, retinal changes, and mortality rates. Of 22 patients with benign HT who had cardiac failure or hypertrophy or enlargement, only six survived five years; the rate of five-year survival in those without T wave abnormalities was considerably greater. The five-year survival rates on the basis of retinal grades were 75 per cent for grades O–I, 56 per cent for grade II, and 15 per cent for grade III. No patient with grade IV retinal-changes survived more than two years. Burgess (60) describes 100 patients who had had HT for at least eight years but were otherwise in good health and had no evident vascular complications; those patients who were age 50 or older when first observed lived out their life expectancy Ungerleider (61) finds that among subjects with identical levels of casual BP, mortality rates were doubled in those who presented electrocardiographic evidence of hypertrophy. Deaths from diseases of the heart were 150 to 200 per cent more frequent than in comparable individuals who had two relatives who died of cardiovascular renal disease prior to the age of 60.

Pierson & Hoobler (62), in a 10-year follow-up of 71 "non-malignant" hypertensives under the age of 50 without other major complications of HT disease, state that a single attack of focal, transient encephalopathy is likely to be followed by fatal recurrence in 30 per cent of the cases within 5 years and 45 per cent in 10 years. Approximately 25 per cent died of other HT complications in these same time intervals, suggesting that a cerebral lesion often indicates the coexistence of vascular lesions in other target organs. Five-year cerebral vascular mortalities were 5 5 per cent in 36 cases benefited by sympathectomy, and 22 per cent in 60 cases not benefited, suggesting that those patients whose DP fell 20 mm. after operation were partially protected against an early death.

rat experiments as nonspecific, unrelated to HT, and possibly corresponding to periarteritis nodosa The clear relationship of the vascular lesions to the degree of HT indicates that they are not the equivalent of lesions of periarteritis nodosa.

Much attention has been given to cerebral vascular disease, particularly in HT where it is a common cause of death. The importance of focal and gross cerebral insufficiency in predicting a subsequent fatal cerebral accident has been attested to by a number of authors, but despite intensive studies there is still no way of foreseeing the occurrence of the initial stroke. In the excellent study of Hudson & Hyland (82) on 100 fatal cases of hypertensive cerebral vascular disease, the actual cause of death was not predictable from the clinical history, except that most patients who had strokes as initial symptoms subsequently died of cerebral vascular accidents.

The application of modern knowledge of circulatory physiology of the brain to the sick patient is discussed in an excellent review by Scheinberg (83). Considerable interest has been shown in the problems of intermittent cerebral insufficiency, and in the value of determining retinal arterial pressure (84) Of seven patients with differences in retinal artery pressures of the two eyes greater than 15 per cent, six were found to have obstructing lesions of the internal carotid artery by arteriography (85).

The role of HT in accelerating atherosclerosis has continued to receive attention and such an effect seems to be an established fact. Viewed thus, HT is never "benign" Experimental studies, in animals as well as man, clearly show that HT, particularly when associated with hypercholesterolemia, results in increased incidence of atherosclerosis (86, 87). Lober (86) shows that in the fourth and fifth decades, the incidence of atherosclerosis is considerably greater in HT than in normotensive subjects One of the best prospective studies is that of Dawber and his associates (88) in the Framingham project who, in a four-year follow-up of a group of men aged 45 to 62, showed that the risk of new coronary episodes increased progressively with increasing levels of BP Men with BPs greater than 140/90 mm Hg and normal cholesterols had three- to sixfold higher incidences of new atherosclerotic episodes during the four years. The total number of patients who have developed coronary occlusion is relatively small If the trend continues, it will unequivocally demonstrate the importance of even slight increases in BP and constitute a strong basis for lowering BP before the appearance of obvious vascular complications Further follow-up by the Framingham group will be awaited with considerable interest

The relative absence of cardiac disease in the Bantu, Japanese, and Filipinos, despite the presence of HT, may be explained by their low serum cholesterol levels So-called Western levels of "normal" serum cholesterol may be abnormal in the presence of HT, thus accounting for the high correlation between HT and coronary disease

A study of HT and arteriosclerosis in 1000 executive and non-executive

per cent of the autopsy series and 21 per cent of resected adrenals (5 adenomas with hyperaldosteronism and 16 pheochromocytomas). This incidence of adenoma is tenfold that found in the control series of normotensive individuals, and should stimulate further research into the role of the adrenal in HT.

The importance of comparing clinical and autopsy data is reflected in the paper by Uys (36), reporting nephrosclerosis in only 2 per cent of 3000 autopsies in Bantus in contrast to the 30 per cent incidence of HT reported by Schrire (29). The reason for this discrepancy is not clear; it also conflicts with the data of Fraser (35).

Wilson *et al.* (79), in an excellent experimental study, irradiated one or both kidneys of rats and noted the onset of HT four to nine months later. Hypertension was curable if the irradiated kidney was removed at that time. Lesions in the non-irradiated kidney were those of HT and distinctly different. In some instances, irradiated kidneys removed at the onset of HT disclosed no microscopic vascular lesions and no interstitial fibrosis. It appears, then, that damage to a kidney by radiation may result in HT without microscopic vascular lesions One wonders whether the irradiated kidney would be negative histologically to electron microscopy and whether the interstitial reaction to irradiation may lead to vasoconstriction of renal vessels. More precise renal functional studies seem indicated.

Hypertension and Vascular Disease

The relationship between the elevated BP and the associated vascular disease has been a controversial subject for many years Some authorities, such as Goldblatt (76), believe that the primary lesion occurs in the kidney and that vascular lesions precede the elevated BP, although the nature and mechanism of such lesions are obscure Others believe that elevated BP is primary, possibly induced by a combination of factors including emotional stress, and that the elevated BP then damages the vessels, producing vascular disease (11, 79, 80).

Deming *et al.* (81) induced HT by administration of deoxycorticosterone acetate and salt or by renal artery constriction in rats on an "atherogenic" diet containing added cholesterol, cholic acid, and thiouracil The rats developed accelerated atherosclerotic changes which correlated with the concentration of cholesterol in the serum, a positive correlation was also found between BP and degree of hypercholesterolemia Masson and his colleagues (80) showed that when HT was produced in rats by partial renal infarction and uninephrectomy, subsequent reduction of BP by administration of hydralazine in drinking water resulted in healing of the acute vascular lesions found in the majority of animals with untreated HT of short duration The lesions were almost completely prevented by maintenance of BP within a normal range by hydralazine, but they reappeared when withdrawal of hydralazine allowed the pressure to rise again. These experiments apparently disprove Goldblatt's interpretation of the vascular lesions in previous

that renal function may be normal when papilledema is present (97, 98, 99). Renal excretory failure follows the appearance of the malignant phase in many cases, but does not necessarily cause it. Further, when the malignant phase is reversed by lowering the pressure, renal function often improves or does not deteriorate further (100, 101). Improvement in the histologic appearance of the renal vascular lesions following treatment has been demonstrated (102).

Kincaid-Smith *et al.* (103), in a study of 197 cases of malignant HT, doubted that the arteriolar necroses resulted from the mechanical effect of extreme elevation of arterial pressure. They found considerable overlap of BP in malignant and non-malignant cases, and noted that SPs were under 200 mm. Hg at the time of diagnosis in 6 of Volhard's original 32 patients. They agree that the malignant phase may complicate all varieties of pre-existing HT, and that it would be unwise to assume that damage to the arteriolar walls is the only mechanism for producing arteriolar necrosis. They conclude that malignant HT is determined by new and unknown factors superimposed upon a high level of BP. Papilledema and retinitis cleared without treatment in three well-documented cases. Papilledema was frequently present when the spinal fluid pressure was normal, confirming the observation of Schottstaedt & Sokolow (97). In one case, papilledema and retinitis were intensified while the spinal fluid pressure was falling. Taylor and his associates (104) also confirm the lack of correlation between DP and cerebral spinal fluid pressure in HT patients without papilledema, but found a significant correlation, though not sufficient to indicate causation, in malignant HT They conclude that papilledema and increased spinal fluid pressure are specific manifestations of HT disease, unrelated as to cause and effect. Brust (105) illustrates the retinopathy of accelerated HT in 108 photographic color plates Occasional discrepancies were noted between the progression of retinopathy in spite of modification of the BP and regression of fundal changes without lowering of BPs. He does not refer to the possibility that the apparent discrepancy is caused by reliance on casual BP readings. Clarke & Murphy (106), in analyzing the neurological aspects of the malignant HT described by Kinkaid-Smith *et al.* (103), found involvement of the nervous system in 79 of 190 cases, most of these patients had an acute vascular episode—focal ischemia in 40 per cent, intracerebral hemorrhage in 38 per cent, and subarachnoid hemorrhage in 5 per cent

Treatment of Malignant Hypertension

The universally poor prognosis in patients with malignant HT was confirmed by Schottstaedt & Sokolow (97) and by Kincaid-Smith *et al* (103): untreated, about 80 per cent of patients with HT and papilledema will die within a year. Data are now available to indicate that in patients with satisfactory renal function, adequate control of the BP with ganglionic

personnel of comparable age and sex showed a higher incidence of HT in males than in females and in non-executives than executives, as well as a higher incidence of arteriosclerosis in non-executives than executives (89). This paper refutes the belief that executives are invariably more prone to HT and arteriosclerosis.

Evaluation of aging of arteries in relationship to HT, independent of vascular complications, has been attempted by utilizing the depressor action of amyl nitrite and its effect on pulse pressure (90).

Electrocardiography and Left Ventricular Hypertrophy

The importance of recognizing the earliest manifestations of left ventricular hypertrophy in hypertensives has been appreciated by a number of investigators. Prognostic studies cited previously (16) have shown that the presence of left ventricular hypertrophy adversely affects prognosis, *and therefore may constitute an indication for potent hypotensive therapy* As a result, the criteria for left ventricular hypertrophy in the electrocardiogram have been restudied with a view to verifying their accuracy and reliability (91, 92). Of 101 patients in whom high voltage was the only electrocardiographic abnormality, 95 were found to have a condition increasing *the load of the left ventricle; 72 of these patients had HT (93).* The rarity of high voltage as a sole abnormality in autopsied cases of left ventricular hypertrophy noted by Griep (94) is not surprising, since high voltage of the QRS complex is an *early* sign. The main value of the early pattern of left ventricular hypertrophy is in the assessment of patients with HT who *have not yet developed severe cardiac enlargement. Individuals with high* voltage of the QRS complexes or the earliest sign of left ventricular hypertrophy have an adverse prognosis compared to the population at large (95). One should always consider the possibility of error when left ventricular hypertrophy is diagnosed solely on the basis of high voltage of the QRS *complex, especially in, young, debilitated, or thin individuals* The presence of high voltage in the electrocardiogram in a patient with HT, however, should lead the physician to suspect that early left ventricular hypertrophy has developed, and this will be important in influencing the decision regarding treatment with potent therapeutic agents

Malignant Hypertension

Controversy still rages with respect to the mechanism of the malignant phase of primary HT Pickering (1) considers the abrupt onset of high DP to be an important pathogenetic factor in precipitating the accelerated phase. Perera *(96) documents a variety of reasons to support his belief* that the malignant phase represents a qualitatively different condition. Goldblatt (76) believes impaired renal function is a prerequisite to the development of the malignant phase. He does not refer to studies which show

through normal kidneys (118, 119); a similar effect is noted in renal HT dogs, but less regularly (120) Experimental renal HT persists after total nephrectomy in dogs and rats (121, 122). It has been demonstrated in the rat and rabbit with chronic renal HT that release of the clamp on the renal artery is followed by complete reversal of HT. Floyer has reviewed this work (122) and postulates that the clamped kidney secretes a substance which is not itself a pressor substance but which acts on the opposite normal kidney in some way, interfering with its BP-regulating activity. The most exciting piece of human evidence in support of the concept that the normal kidney acts metabolically to maintain normal BP is the successful homotransplantation of one normal kidney from a twin to his mate with bilateral kidney disease, uremia, and HT (123) Following transplantation there is a significant fall in BP which, however, is generally not complete until both diseased kidneys have been removed.

The major fact which emerges more and more clearly is that an essential non-excretory function of the normal kidney is the regulation of BP. Highly interesting data are accumulating on the presumed secretory function of the juxtaglomerular cells and its possible relationship to pathogenesis of experimental HT (124, 125)

Neural factors in hypertension.—The superficially plausible and attractive hypothesis that excess sympathetic tone alone is responsible for the increased peripheral resistance in essential HT remains unsupported by direct evidence (126) Urinary excretion of norepinephrine is normal in human HT (127, 128). In experimental animals peripheral resistance and cardiac output can be increased by stimulation of certain areas of the hypothalamus, but similar data in man are not available. Crandall *et al* (129) have induced HT in dogs by damping carotid sinus pulsations, but ligation of the carotid arteries distal to the sinus along with the occipital arteries produced the same result McCubbin *et al* (130), in seeking neurogenic mechanisms that might contribute to the maintenance of chronic renal HT, have shown that the baroreceptors are "reset" at the HT level of pressure and act in completely normal fashion to preserve this level. Similarly, when HT is "cured," the receptors adjust again to normal BP. Both experiments suggest that the baroreceptors are less powerful than other vasoregulatory mechanisms Heymans (131) has explained the phenomenon of resetting by indicating that the direct stimulus to baroreceptor activity is not intra-arterial pressure but the degree of stretching of the sinus wall itself He postulates that in chronic HT, tension of the arterial wall somehow decreases, and thus the baroreceptors react normally to changes in BP at the HT level The neural control of arteries was reviewed by Folkow (132), and regulation of peripheral resistance has been recently summarized (3, 133). Evidence is presented that anatomical changes in arterioles are secondary to the elevated BP and not primary (134).

Water and electrolyte metabolism in hypertension.—The exact role of

blocking agents results in approximately 40 per cent survival at the end of three to five years (3, 4, 107, 108, 109). All authors have commented on the adverse prognosis of patients with renal insufficiency. Perry & Schroeder (101) showed that if BP is reduced gradually, increased survival is possible, even in patients in the malignant phase complicated by azotemia The mortality in the azotemic group after four years was 69 per cent, compared with only 36 per cent in those without azotemia. The importance of patient cooperation is underscored by the fact that the four-year mortality of 178 patients who followed treatment was 24 per cent, whereas in 27 patients who discontinued treatment it was 78 per cent. The rarity of deaths from heart failure was also stressed

In evaluating therapy, one of the earliest signs of improvement is reversal of neuroretinopathy. The rarity of spontaneous reversal is documented by Keith & Wagener (110) and by Schottstaedt & Sokolow (97). The former authors noted that unexplained reversal of retinopathy occurred in only 15 patients during a 20-year period

Surgical treatment of the malignant phase has been reported by a number of authors. In Hoff's (111) five patients with malignant HT and good renal function, adrenalectomy effected no significant changes in BPs, but papilledema improved in all cases. Four of the patients died within 20 months of operation. Interestingly, his Case 3, a 26-year-old man, had a small right kidney and an aortogram showed a decrease in blood supply. Adrenalectomy, rather than nephrectomy, was performed; today a nephrectomy or renal endarterectomy probably would have been done. Arnott *et al.* (112) described the effects of total adrenalectomy in six patients with HT, five of whom had papilledema. Three of the five died within a few months; the other two were alive 12 and 18 months, respectively, after adrenalectomy Zintel and his associates (113) reported the results of a three- to seven-year follow-up of 76 patients with HT treated with thoracolumbar sympathectomy. One-half of the patients were in the malignant stage and 49 per cent of them died.

Experimental Hypertension

Renal and renoprival hypertension.—Hypertensin II, a recently synthesized octapeptide, has been shown to be the active vasoconstricting substance in renal HT (114, 115). By mutual agreement it is to be called "angiotensin"

The fact that passive transfer of hog antirenin to dogs with chronic renal HT causes a significant fall in BP is considered the only valid evidence that renin participates in the HT process (116) Grollman *et al.* (117) have postulated that renal HT is not caused by liberation of a pressor agent,

arterial walls influence reactivity, but this has not been demonstrated in human essential HT (154, 155).

Monoamine oxidase inhibitors—Both serotonin and norepinephrine may undergo oxidative deamination via monoamine oxidase (MAO) activity. Remarkably, however, isopropyl isonicotinyl hydrazine inhibits this activity yet induces postural hypotension (156), the degree of which parallels the suppression of serotonin conversion (157). Since effective inhibition of MAO activity does not cause the further elevation of BP that might be expected if norepinephrine were metabolized predominantly via MAO, Mendlowitz *et al.* (158) have suggested that the other known pathway of norepinephrine degradation, namely by the enzyme O-methyltransferase, may be abnormal in human HT.

Hollander & Wilkins (159) reported unimpressive results from the use of iproniazid on 51 patients with arterial HT. Gillespie (160), utilizing JB 516 (1-phenyl-2-hydrazino-propane) in 21 patients, observed orthostatic lowering of pressure in 18 and significant BP reduction in recumbency in six. Six patients experienced temporary loss of red-green color discrimination. Both promote the depressor action of serotonin on BP but do not affect sensitivity of digital arteries to norepinephrine. Both are potentiated by chlorothiazide. Their mechanism of action is unexplained and may not relate directly to inhibition of MAO The most unusual effect on color vision is potentially a fertile lead for retinal physiologists.

Serotonin (5-hydroxytryptamine).—The present status of serotonin, clinically and experimentally, has been reviewed (161, 162) Its action upon blood vessels is dependent on many other physiological variables. In man, depending upon the rate and amount of injection, serotonin may lower or raise BP; usually it does both in sequence. In animals, there are significant species differences in response, but uniformly there is marked constrictor effect upon renal vessels, even to the point of tissue necrosis At present, serotonin has no known clear-cut role in either the normal or HT state.

Adrenal factors in hypertension—Despite the usual "cure" of HT when an adenoma of the adrenal is removed, the role of aldosterone in producing HT is not clear Hypertension may be absent in some cases of hyperaldosteronism despite the usual electrolyte derangements (136); the urinary excretion of the hormone in HT hyperaldosteronism is usually less than in secondary aldosteronism or when Na^+ is markedly restricted in a normal person (163); long-term administration is necessary to induce HT in rats and this is not influenced by Na^+ (164) Proof is lacking that aldosterone participates in the pathogenesis of human essential HT. The subject has been thoroughly reviewed (165, 166, 167).

Secondary Hypertension

Renal artery occlusion—One of the major developments within the past five years has been the discovery of the importance of atherosclerotic renal

Na^+ in the production and maintenance of experimental HT has not been defined, but a summary of the present position has been made by Ledingham (135). While the influence of K^+ depletion on BP cannot be assessed, evidence indicates that the action of Na^+ is independent of adrenal cortical hormones, even though there is no regular pattern of electrolyte disturbance in renal, renoprival, or steroid HT. The intake of Na^+ is not the sole determinant of HT, *but in the presence of disturbed homeostatic mechanisms* experimental animals become extremely sensitive to changes in electrolyte intake, just as do patients with HT who have undergone adrenalectomy. Since changes in distribution of body water and cellular electrolyte content may alter membrane potential and contractility either of the myocardium or of the smooth muscle of arteriolar wall, Ledingham postulates that this could be the link with HT, but at present it must remain purely hypothetical.

Conn (136) has stated that the body content and distribution of Na^+, K^+, and water are identical in primary aldosteronism associated with HT and in periodic paralysis without HT. Winer (137) has found normal values for body electrolytes and water in essential HT, confirming one report (138) and contradicting another (139) It has been amply reconfirmed that HT patients excrete a greater proportion of infused Na^+ in solution than do normals, but the significance of this is unknown (140). This phenomenon is partially reversed by anti-HT therapy (141). Tobian & Redleaf *(142) suggest that in HT, edema in the arteriolar walls may account for* the increased peripheral resistance; they have shown increased Na^+ and K^+ content in aortas of rats with experimental HT. It is tempting to use these data in support of the views of Mendlowitz *et al.* (143) and Conway (144). The action of chlorothiazide in potentiating hypotensive effects of other drugs could also be explained on the basis that it reduces the arteriolar Na^+ content and secondarily reduces wall thickness, thereby increasing lumen size (145).

Na^+ may also influence BP by its effects on plasma volume, as the work with chlorothiazide suggests. The potentiating action of chlorothiazide is lost if the plasma volume is replaced by dextran, even if Na^+ loss is not replaced (146).

Vascular reactivity.—Patients with established HT exhibit abnormal increases in peripheral resistance or decrease in peripheral flow in response to epinephrine or norepinephrine (147, 148, 149). Hydrocortisone and ACTH induce similar responsiveness in non-HT persons (150, 151). This apparent hyper-responsiveness does not necessarily indicate a greater degree of arteriolar contraction because it may result from the fact that the caliber of arterioles is already reduced by thickening of the walls caused by edema, hypertrophy, sclerosis, or all three (152, 153). Thus, a normal amount of arteriolar contraction could cause a disproportionately great increase in resistance. Changes in electrolyte concentrations and in tension of

Howard test. These authors found focal renal lesions in 256 HT patients who had aortography during a 3½-year period. Improvement in DP occurred in about 75 per cent of the operated cases. They recommend renal angiography in the following instances: (*a*) disparity in measured length or excretory function of the kidneys as revealed by intravenous pyelogram; (*b*) HT without evident cause in a patient under 35; (*c*) malignant HT in a patient over 55; (*d*) non-familial HT of recent onset in any patient with rapid progression into the malignant phase; (*e*) HT which develops or becomes worse following an attack of flank pain which may represent infarction of a part of a kidney. Additional reports confirm the importance of renal artery occlusion (174 to 178). The techniques and complications of aortography have been described in detail (179, 180).

As a screening technique for renal artery lesions, Winter (181) has devised a renogram recorded from scanning the circulation of intravenously injected I^{131} through the individual kidneys This correlates well with other tests of renal function but does not yield direct information about the presence or absence of a renal artery lesion

this relationship, noting the irregular association of kidney disease with vascular disease and HT, the fact that pyelonephritis may terminate in uremia with normotension, and that HT may precede or follow the onset of renal insufficiency. He states that we urgently need additional information on the relationship of unilateral kidney disease to HT since pyelonephritis with advanced vascular changes may or may not be associated with HT Part of the difficulty with respect to pyelonephritis concerns the need for more precise methods of recognizing chronic and asymptomatic states during life. Data regarding this and reviews of the modern management of pyelonephritis are available (183 to 187).

Pathological criteria are not completely established, and the genesis of the vascular lesions in regions of pyelonephritis is not understood Chronic pyelonephritis was found in 41 of 71 kidneys removed surgically for unilateral renal disease, 35 per cent of those with pyelonephritis and 23 per cent of those without pyelonephritis had been associated with HT (187). Brod (188) found a 60 per cent incidence of HT in a group of patients with pyelonephritis, compared to 15 per cent in patients with chronic cholecystitis. When a family history of HT was present, the figures rose to 85 and 30 per cent, respectively.

Woods' (189) data show that rats with hormonal HT are more prone to experimental pyelonephritis He hypothesizes that various injuries to the kidney, including nephrosclerosis, may predispose one to pyelonephritis In this instance, pyelonephritis may be a secondary condition superimposed on HT disease This would explain in part the unexpectedly high

artery lesions in the production of HT, even though Blackman, in 1939, had shown that 86 per cent of HT patients had arteriosclerotic plaques in a renal artery at autopsy in contrast to 11 per cent of controls (168). Recognition that this is probably the most common cause of severe HT in older individuals comes approximately 25 years after the pioneer experimental work of Goldblatt. Connor *et al.* (169), in a study of 70 cases of HT, noted that those patients whose BP improved following nephrectomy invariably had a decreased urine volume and Na^+ concentration on the involved side compared to that of the opposite side. Patients who did not improve after nephrectomy did not show these changes, nor did patients who had bilateral renal disease This paper led to the so-called Howard test for recognizing obstructive lesions of the renal artery, which revitalized interest in the possibility of recognizing renal artery lesions without aortography. Meticulous attention to technical details is essential.

Impetus for the use of aortography in the recognition of occlusive disease of the renal artery came from Poutasse & Dustan (170), who described 104 selected HT patients subjected to aortography during the previous two years As clues to the presence of renal artery lesions they stressed the importance of abdominal or flank pain, followed by the rapid development of severe HT in a patient with a negative family history of HT. They found 30 individuals with localized lesions, 19 of whom were subjected to nephrectomy or corrective renal artery surgery Five of seven patients followed for more than a year had consistently normal BPs and two had remission of the malignant phase They recommended nephrectomy when the involved kidney shows distinct atrophy and reduced function or, when possible, segmental endarterectomy or renal artery corrective surgery Floyer's work (3) emphasizes the importance of restoring normal blood flow to the kidney. The chance of reversing HT and preserving normal kidney function warrants the extra risk involved in renal endarterectomy. An exact human counterpart of Wilson & Bryom's classic experiments in rats (171) has been reported in an eight-year-old girl with malignant hypertension (172) Left nephrectomy was performed because of reduced dye excretion on the left, and no nephrosclerotic changes were noted in the kidney. At autopsy following her death from uremia nine days later, the stump of the left renal artery was found nearly occluded near the aorta and the right kidney showed severe HT vascular changes.

Poutasse & Dustan (173) compared aortography with differential water and electrolyte excretions by the Howard test. Results of the two tests concurred when there was obstruction of the main renal artery. With obstruction in a branch of the main renal artery, or pyelonephritis, however, the glomerular filtration rate, volume, and osmolarity decreased on the affected side, but Na^+ excretion remained equal on the two sides This explanation of the difference between branch and main renal artery lesions is a valuable contribution and explains many apparent discrepancies of the

urinary catechol amines may be missed in the paroxysmal form; therefore in such instances three to five blood samples should be obtained at 2-min. intervals following induction of an attack by histamine.

Polycystic kidney—An excellent monograph on bilateral polycystic disease of the kidneys, a follow-up of 284 patients and their families, has been published by Dalgaard (204).

Treatment of Essential Hypertension

Experience has amply confirmed Smirk's optimistic view in his 1955 review (205) that potent drugs have at last resulted in effective treatment of the disease. A number of papers emphasize the difficulties in the clinical evaluation of anti-HT drugs Corcoran, Dustan & Page (206) discuss the problem of estimating the severity of HT disease and indicate a need for alternate courses of drugs interspersed with placebos. Weekly mean pressures, derived from pressures recorded twice daily by the patient or a member of his family, were found to be a valid representation of the week's readings and were comparable to similar means obtained by nurses in the hospital. They prevent error in interpretation based on reliance on uncontrolled casual readings They state that only 1 per cent demonstrated psychological stress from home readings Shapiro (207) discusses the problem of evaluating drugs in the treatment of HT, emphasizing the non-pharmacological variables which contribute to HT effects, symptomatic improvement, and side effects He discusses at length experimental design and the need for control periods during and after therapy, as well as before. Goldring and associates (208) have proved the effectiveness in reducing BP of calculated and deliberate dramatization of a regimen of reassurance to the patient They obtained striking falls in pressure which returned to control levels in eight weeks or less after cessation of treatment, showing that nothing more than a transient effect on pressure had been achieved Perera (9) discusses therapeutic principles and objectives of treatment of hypertensive vascular diseases If the lives of some patients in the accelerated phase are prolonged by therapy, is one justified in assuming that a reversal of the pathological process has occurred? It is established that healing of necrotizing lesions follows anti-HT therapy (80, 102); Moyer *et al* (100) demonstrated arrest of progressive renal deterioration when BP was lowered with ganglionic blockers. Disappearance of proteinuria indicates that glomerular lesions are actually being reversed with treatment Freis (209) discusses the discrepancy between home and office readings of BP, pointing out a need for utilizing home readings in the evaluation of therapy. This is essential in order to avoid overdosage. Rosenheim (210) also emphasizes this point, and utilizes short overnight visits to the hospital in lieu of home readings. Stewart (15) formulates a set of principles as a guide to the treatment of HT with new HT agents. He believes that HT disability and death are caused by vascular lesions, that arbitrary reduction of BP can delay or arrest

incidence of pyelonephritis in HT patients in whom earlier thorough studies had disclosed no evidence of pyelonephritis. This merits further study.

Merriam *et al.* (190) found that HT patients with chronic pyelonephritis, compared with a larger group without pyelonephritis, had slightly increased mortality and significantly higher average DP for the same grade of arteriolar sclerosis Other papers (191, 192) discuss the role of pyelonephritis in HT.

Primary hyperaldosteronism.—The clinical syndrome defined by Conn, earlier described as "potassium-losing" nephritis, is now recognized as a major cause of HT (136, 163, 193, 194). Clinical features include variably severe muscular weakness, tetany, paresthesias, polyuria, polydipsia, HT without edema or signs of Cushing's syndrome, together with hypokalemia, hypernatremia, alkalosis, alkaline urine of low specific gravity, mild proteinuria, high urinary aldosterone and normal 17-hydroxycorticoids and 17-ketosteroids (193). Any or all of these may be absent, edema may occur (195), and urinary aldosterone may be normal if the patient is severely depleted of body K^+ (163). Bartter & Biglieri (196) have proposed Na^+ restriction to 250 to 500 mg. per day as a simple yet reliable means of determining whether a patient with HT and hypokalemic alkalosis has hyperaldosteronism; serum K^+ should rise, urinary Na^+ should fall. Electrocardiographic signs of hypokalemia in a HT patient should alert one to the possibility of this diagnosis if the use of chlorothiazide and laxatives has been excluded. Methods for determining aldosterone in the urine are being perfected, but at present are cumbersome and not entirely reliable. Surgical removal of an adenoma (rarely hyperplasia or carcinoma) usually results in remission of HT unless advanced renal disease has developed; even in its absence, however, HT has persisted postoperatively after all electrolyte disturbances have been corrected (136).

Cushing's syndrome—The severity of HT occurring in patients with Cushing's syndrome has been emphasized (197) Montgomery & Welbourn (198) have reported the return of BP to, or near, normal in 12 of 13 patients following adrenalectomy whether they received substitution therapy or not; four of these had malignant HT The mechanism whereby excessive secretion of the so-called glucosteroids by the adrenal cortex induces HT remains unknown.

Pheochromocytoma—Kvale *et al* (199) have reviewed 50 cases, and Roth and his associates (200) have thoroughly discussed the details of diagnostic tests, with considerations of misleading positive or negative results. Von Euler and & Strom (201) emphasize the value of determining urinary catechol amines but admit the difficulty of knowing the upper limits of normal. Hoobler *et al* (202) outline a "routine screening procedure" and state that only when angina makes pharmacologic tests dangerous should urinary catechol quantitations be done first. Reutter and his associates (203) make an important point: a diagnostic rise in blood or

have become normotensive. With prolonged treatment, control of BP became more complete and side effects less prominent. Their paper furnishes detailed discussions of individual patient responses, adjustments in dosage, and helpful measures for the control of side effects. Cottier *et al.* (225) found good clinical effects and noted only slight reduction in renal plasma flow, glomerular filtration rate, and Na^+ clearance in half his patients. Freis & Wilson (226) reported that the greater potency and longer duration of action of mecamylamine were advantageous but that the addition of tranquilizers or chlorothiazide equalized the clinical value of all currently used ganglion-blocking agents Mecamylamine produces parasympathetic blockade, but its use has eliminated sudden unpredictable falls in pressure and sudden development of ileus When such occur, they are the result of increased dosage, not of unexpected increase in intestinal absorption. Nonetheless, mecamylamine in high doses has produced gross tremor, choreiform movements, depression, agitation, hallucinations, or convulsion in some patients (227). These effects subside within a few days to two weeks upon withdrawal of the drug or reduction in dose and are attributed to diffusion of the drug into the brain.

Pempidine, a tertiary amine and a derivative of piperidine, is pharmacologically similar to mecamylamine (228). Following an oral dose, BP falls within an hour, occasionally after 3 or 4 hr ; the maximum response lasts 1 to 3 hr , and total duration of effect is usually 6 to 7 hr. In patients on treatment, four equally spaced daily doses are generally needed. Harington *et al.* (229) report an average daily maintenance dose of 32 5 mg. of the bitartrate, with initial doses of 2.5 mg. four times daily, which can be rapidly increased to higher levels As with mecamylamine, no significant tolerance has been noted. Whether pempidine and mecamylamine, by virtue of their unique structure, act differently from the quaternary amines remains uncertain; the problem is reviewed by Paton (230).

Mechanism of action of ganglion-blocking agents—Depending upon the technique used, ganglion blockers have been reported to reduce peripheral arteriolar resistance (231), reduce venomotor tone and, secondarily, cardiac output by pooling of blood in the splanchnic bed (232, 233), reduce both arteriolar and venous tone (234), and reduce cardiac output by direct action on the heart (235). In the presence of heart failure, however, these agents increase output, since reduced ventricular filling permits more efficient contraction of overstretched ventricular muscle fibers (236).

Chlorothiazide and derivatives.—Chlorothiazide, when administered to HT patients without heart failure, induces a negative Na^+ and Cl^+ balance, a reduction of the extracellular fluid space by approximately 2000 ml, and commensurate weight loss (237, 238, 239) The full effects appear within three days' therapy with doses of 0 5 to 1.0 gm and are associated with reduction averaging 15 per cent of the mean BP. Much more striking and unexpected was its remarkable synergistic effect with other anti-HT agents,

these lesions and that relatively short falls in pressure may suffice. He stresses the point that even slight rises in pressure result in mortality rates from cardiovascular-renal disease of nearly four times average.

ANTIHYPERTENSIVE DRUGS

Smirk (205) and Yonkman (211) have reviewed the early literature on anti-HT agents. The hope that the separation of protoveratrine into its two components would lead to the development of a preparation possessing highly desirable therapeutic properties while lacking its unfortunate side reactions of weakness, nausea, and vomiting, was short lived (212). There are no oral "preparations of choice," and their use remains restricted to the few who tolerate them well and to those who cannot be managed satisfactorily with other therapy Protoveratrine remains a useful and valuable agent in the treatment of toxemia of pregnancy (67).

The effectiveness and reliability of reserpine in the treatment of HT emergencies, including HT encephalopathy, by parenteral injection is well established (213) The maximum dose needed for full effect varies from 2 5 to 5 mg, acts usually within 3 hr. and lasts an average of 10 hr., but doses may be given every 4 to 12 hr. according to the individual patient's response. The fall in mean pressure averages 20 mm. It has been effective in HT secondary to acute and chronic nephritis in doses of 70 to 150 µg./kg body weight, alone or combined with hydralazine (214).

Additional ganglion-blocking agents have been introduced and found comparable to hexamethonium and pentolinium in clinical pharmacology and general usefulness: chlorisondamine (215, 216), pentacynium *bis*-methylsulfate (217), trimethidinium methosulfate (218, 219). Mecamylamine and pempidine have been shown to have different pharmacologic properties and generally more desirable clinical effects

Mecamylamine (3-methylamino-isocamphane hydrochloride), a secondary amine, diffuses readily into cells, is almost completely absorbed following oral administration, acts for a significantly longer period, and induces almost negligible tolerance (220, 221, 222). Reports of its clinical use uniformly note greater ease of maintaining smooth BP responses because of its longer anti-HT action and predictable effects (223 to 226). Smirk & McQueen (223) recommend 5 mg orally as an initial dose or titration of each patient by intravenous administration of 0 5 mg of mecamylamine per minute with continuous BP recording as the best guide to initial dosage They give the drug twice daily, with a smaller supplemental dose at 2 P.M. and a 30 per cent larger night dose About 50 per cent of their patients had good results; a third had distressing side effects of parasympathetic blockade. The average daily dose was 33 mg. Moyer *et al.* (224) begin treatment with 2 5 mg twice daily, gradually increasing to four doses daily, but omitting bedtime doses in one-fourth of their patients Sixty to 70 per cent have responded to average daily doses of 29 mg., and 30 to 40 per cent

asymptomatic HT patients without vascular complications will have increased survivals if their BPs are lowered or whether such treatment will prevent or delay appearance of vascular complications. Since the duration of life of the asymptomatic patient averages 20 years, it will be necessary to do a controlled clinical trial to answer this last question. The problem of evaluating the influence of BP on atherosclerotic complications is difficult because the clinician can only recognize *complications* of atherosclerosis, not atherosclerosis per se. Since life expectancy quite clearly increases following treatment of most of the severe aspects of HT, it appears reasonable that treatment of established HT at an earlier stage should be considered desirable.

Evidence for increased survival in patients with the malignant phase has been documented (3, 4, 107, 108, 109). All investigators agree that, in contrast to almost zero survival of untreated patients after two or three years, 40 to 50 per cent of patients with malignant HT and satisfactory renal function will survive five years if BPs are adequately lowered Reversal of the malignant stage can occur whether the patient has essential HT and is treated with ganglionic-blocking agents, sympathectomy, or adrenalectomy, whether he has pheochromocytoma (256), primary aldosteronism (257) or Cushing's disease (198) and is treated by removal of the adrenal tumor or adrenal resection, whether he has unilateral pyelonephritis or unilateral renal artery occlusion (173, 178, 211) and is treated by either nephrectomy or surgical repair of the renal artery. The importance of satisfactorily controlling pressure was demonstrated by Perry & Schroeder (107) who noted in 82 patients with malignant HT that mortality in four years was 100 per cent when DP was uncontrolled by therapy, was 60 per cent when DP was partially controlled, and 31 per cent when DP was adequately controlled. Regardless of the cause of HT, lowering of the BP results in reversal of the malignant phase. Similar benefits accrue in cardiac failure. Shirley Smith & Fowler (258) noted that during the period of therapy no patient developed heart failure and no deaths occurred due to heart failure Goldring & Chasis (259) found life expectancies of 1 8 years following the first evidence of heart failure in a survey of 138 cases of fatal HT In contrast, in 310 HT patients treated from two to seven years by Smirk *et al.* (260), 78 per cent with left ventricular failure and 64 per cent with combined failure were alive three years after the onset of hypotensive therapy. These results are even more impressive because significant dyspnea on exertion was present in 127 and cardiac failure occurred in 85 prior to therapy Heart failure was the sole cause of death in only 19 patients (about six per cent) of the total series Smirk described prompt and often dramatic relief of HT cardiac asthma following intravenous hexamethonium, disappearance of pulsus alternans and gallop rhythm, and decrease in size of the heart and improvement in the electrocardiogram following lowering of BP. Freis & Wilson (261) demonstrated the same benefit with respect to cardiac failure: de-

especially the ganglion-blocking drugs, dosage of which often must be reduced 50 per cent when chlorothiazide is added to the regimen (240, 241). The mechanism of this appears to be a reduction in plasma volume rather than Na^+ depletion since replacement of the plasma deficit with Na^+-free dextran promptly abolishes the effect on BP (146). Dustan *et al.* (242) postulate that oligemia induced by chlorothiazide stimulates greater vasomotor tone which, in turn, is more readily responsive to the ganglion-blocking agents than are the usual mechanisms which maintain HT. The details of chlorothiazide therapy, including toxic effects, have been thoroughly described in recent literature (243 to 247).

Hydrochlorothiazide is a more potent form of chlorothiazide in a range of 10 to 20 to 1 on a weight-for-weight basis (248, 249). The effects on renal excretion of water and electrolytes, including K^+, are comparable Spittel *et al.* (250) report that one-third of patients with mild to moderate HT became normotensive on hydrochlorothiazide alone in doses of 50 to 200 mg. daily. Significant falls in serum K^+ occurred in 7 of 18 patients.

A more recent modification of chlorothiazide is the replacement of the chloride by a trifluoromethyl group. The resulting agent, flumethiazide, appears in every way to be comparable to chlorothiazide in its pharmacological actions (251). Data are scanty to date.

Surgical treatment—A number of papers have appeared summarizing results of sympathectomy and adrenalectomy, alone and in combination, in the management of arterial HT (111, 112, 113, 252).

Superior results were noted with adrenalectomy, compared to sympathectomy, particularly with respect to improvement in congestive failure (253). Decrease in the number of sympathectomies performed in recent years is clearly indicated from Smithwick's report of an average of 25/year in 1957–1958, compared to 350/year in 1947 (254). A double-blind study, with matched controls, concluded that only patients with accelerated or malignant HT had an improved 10-year survival following sympathectomy (255).

It seems clear that survival is increased with surgical procedures when papilledema or cardiac failure or both are present Surgical procedures are more radical and have produced no better results than medical treatment in the two-thirds of the patients in the total series in whom the above complications were not present.

Evidence That Increased Survival and Therapeutic Benefits Accrue From Hypotensive Agents

Hypotensive therapy has been in fairly extensive use since 1951. There are now substantial data indicating that lowering BP in HT patients unequivocally increases survival in the most severe forms of HT, notably, malignant HT and in patients who have cardiac failure There are insufficient, but suggestive, data which show that atherosclerotic complications may be decreased There is as yet inadequate evidence to indicate whether

Perry & Schroeder (263) believe that the basic mechanism leading to HT may be reversed if BP is lowered continuously for a sufficient length of time. Of 79 patients who had maintained DPs consistently below 100 mm. Hg, the final doses were only 73 per cent of the initial dosages of the blocking agents, whereas 35 patients whose HT had not been so reduced were still taking 97 per cent of their initial dose. It was possible to discontinue hexamethonium completely in 19 patients, and in 10 patients to discontinue both hexamethonium and hydralazine without a recrudescence of HT. It is possible that with persistent lowering of the BP the baroreceptor "thermostat" may again be lowered to the point where it effectively prevents a rise in pressure above normal with stress or pressor agents, this, if true, would be a most important reason for continuing effective therapy over a period of years

Conclusion

It is now apparent that every patient with HT must be scrutinized for the presence of detectable and treatable causes of elevated BP. Vigorous anti-HT therapy should be instituted in all patients with HT in the malignant phase, in those with cardiac failure, and in those with left ventricular hypertrophy and dyspnea on exertion even if objective signs of cardiac failure are absent The decreased life expectancy in younger individuals, especially males with left ventricular hypertrophy and high basal pressures, cogently suggests that anti-HT therapy should be begun in these patients even though definitive evidence for its value has not yet been obtained. The clear association of HT and atherosclerosis suggests that attempts at lowering the serum cholesterol, as well as the BP, are wise, although the therapeutic value is unproved.

If our understanding of the HT process in man is to advance beyond the speculative state, fruitful investigation of basic mechanisms is in order, notably the mechanism whereby the normal kidney, or normal kidney tissue, acts to maintain normal BP in man and in the experimental animal; the biochemical, physiological, and anatomical response of human arterioles to controlled experimental conditions, and the role of varying types of emotional stress in initiating or aggravating HT

crease in heart size and improvement in the electrocardiogram in 96 patients with severe HT treated with pentolinium. Corcoran, Page and their associates (262) noted that there were no deaths from cardiac failure in 106 patients treated with various drugs; all deaths were due to cerebral or coronary atherosclerosis. Perry & Schroeder (263), in a study of 114 cases treated with hexamethonium and hydralazine, noted that heart failure had almost disappeared, and that only a handful continued to take digitalis.

Improvement in the electrocardiogram following therapy is well documented by Hay (264). The study of Helmcke and his associates (265) indicates that improvement was most common in those who had a satisfactory response to treatment, but this was not consistent Improvement in the electrocardiographic pattern of left ventricular hypertrophy in HT subjects following hypotensive therapy strongly suggests that cardiac hypertrophy in HT is reversible. Decrease in the work load of the left ventricle may logically be considered to delay or to prevent the onset of cardiac failure. Since, as has been documented, cardiac failure can be reversed and prevented with hypotensive therapy, it seems reasonable to begin therapy when left ventricular hypertrophy has appeared, before the development of cardiac symptoms.

The effect of treatment on vascular deterioration in the kidney is described by Moyer *et al.* (100), who performed serial renal clearance studies on 64 patients being treated with potent drugs Comparison of treated and untreated cases showed that effective reduction of the BP arrested renal vascular deterioration in patients with severe and moderately severe HT. No further progression of renal disease occurred over a period of years Abrahams & Wilson (266) noted that in 27 patients with primary renal disease and impaired renal function, no further deterioration occurred when BP was lowered gradually if the creatinine clearance was more than 50 ml/min. and BUN was less than 60 mg. per 100 ml Perry & Schroeder (101) showed that patients with papilledema and impaired renal function could have greatly increased life expectancy if their BP's were lowered gradually, even though they had azotemia

Evidence for reduction in the incidence of atherosclerotic complications is scanty. It is reported that the incidence of arteriosclerotic complications which developed during the course of drug therapy over a five-year period was 16 per cent of 49 patients with good responses, compared to 40 per cent of 57 patients with fair to poor responses (267). The authors concluded that this was partial evidence that arteriosclerotic complications were less if DPs were lowered to less than 110. Clinical, pathological, and experimental data indicate that the accelerated arteriosclerotic changes may be reversible (80, 100, 102). It seems clear that there is insufficient evidence on which to conclude that atherosclerotic complications are reversed or curtailed by hypotensive therapy, but the evidence is suggestive and considerably more study is warranted.

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CARDIOVASCULAR DISEASE: PULMONARY HYPERTENSION[1,2]

By Averill A. Liebow, M.D.
Department of Pathology, Yale University School of Medicine, New Haven, Connecticut

Refined physiological measurements correlated with quantitative anatomical observations have, in recent years, clarified some important problems of the lesser circulation Only a glimpse can be provided in this chapter of the more current of these achievements Meneely (89) has provided an interesting brief historical survey. Several international symposia (2, 112) have brought together much information, and the Nobel prize lectures of Cournand (31) and Richards (95) have been published. "Cor pulmonale," "cardiopulmonary disease," and "pulmonary hypertension" have received more or less extensive treatment (23, 35, 74, 79, 86, 103, 123). Brill (23), in considering semantics, concluded that "cor pulmonale" should be defined as right ventricular hypertrophy, strain, or failure caused by disordered pulmonary circulation; this would include a lesser circulation affected by failure of the left heart. This conclusion seems justified, especially since pulmonary vasospasm can apparently accompany the latter, as in mitral stenosis (MS) (120).

Edwards (39), in a Conner Memorial lecture, provided a synthesis of his views based on his extensive observations of the pulmonary circulation in hypertension. Particularly productive has been Heath in collaboration with Edwards and others (57 to 66) In the effort to determine the prognostic significance of pulmonary vascular lesions, papers of the Evans, Short & Bedford group (40 to 42) have also dealt extensively with pulmonary hypertension of various types. Extensive case studies have also come from German clinics [Köhn & Richter (72, 73); Loogen (85)]. Wood's Croonian Lectures (121, 122) have stated his concept of the "Eisenmenger sydrome" as pulmonary hypertension caused by a high pulmonary vascular resistance with reversed or bidirectional shunt, no matter whether the latter is atrial, ventricular, or at the aorta-pulmonary level Although the propriety of using the eponym can be questioned, it is important to understand the concept. Wood's (123) classification of mechanisms of hypertension into hyperkinetic (from a high pulmonary blood flow), passive (from a high pul-

[1] The survey of the literature pertaining to this review was concluded in July, 1959

[2] The following abbreviations are used in the text: 5-HT [5-hydroxytryptamine (Serotonin)]; IASD (interatrial septal defect); IVSD (interventricular septal defect); LAP (left atrial pressure); MI (mitral insufficiency); MS (mitral stenosis); PAP (pulmonary arterial pressure); PDA (patent ductus arteriosus), PPH (primary pulmonary arterial hypertension).

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brought about by reducing the O_2 content of air ventilating, or blood perfusing, a lobe.

Duke (38) has made another significant contribution in attempting to elucidate the mechanism previously demonstrated by her whereby alveolar hypoxia results in an elevation of pulmonary vascular resistance. In isolated cats' lungs ventilated with nitrogen and under constant volume inflow of blood, there was a pressor response even when the lungs had been chronically denervated and this response was not inhibited by lysergic acid diethylamide, a substance competent to abolish the pressor response to 5-HT in control experiments. No evidence of a circulating vasoconstrictive substance was found by perfusing a denervated hind limb with blood which had been partially deoxygenated in the lungs.

In 11 of 13 patients with MS subjected to acute hypoxia, Yu and coworkers (126) found an increase in pulmonary vascular resistance. The pulmonary arterial pressure and heart rate rose in two-thirds of the subjects and the cardiac index was also increased in approximately 50 per cent.

Since hypoxia and hypercapnia often occur together, Braun *et al.* (20) considered it of interest to investigate the effect on the pulmonary arterial pressure in dogs of respiring 10 per cent O_2 and 10 per cent CO_2 in N_2 Again the response was pressor, but these experiments are weakened by failure to measure the cardiac output.

Liljestrand (80), in continuation of his early studies of the subject, concluded that changes in pulmonary vascular resistance upon ventilating isolated cats' lungs with various gas mixtures could be mediated by changes in pH A decrease in pH of 0 01 unit corresponded to a rise in perfusion pressure of about 1 per cent. This could well account for the variable results of ventilating the lungs with gases under various conditions

Barnard (11) found that in mice subjected to O_2 tensions of 100 mm or less for 35 days there was hypertrophy of the right ventricle, and of both ventricles in animals kept at 95 or 80 mm. tensions for the same length of time

The effects of increasing the oxygen tensions of alveolar air have been studied Besides providing hemodynamic data on 26 healthy ambulatory subjects, Barratt-Boyes & Wood (12) demonstrated that when 95 per cent oxygen is inspired there is a fall in pressure in the pulmonary arteries and right ventricle without a constant change in cardiac output, while the systemic arterial blood pressure rises There is a fall in heart rate, while the stroke volume increases. Swan *et al.* (115) found that in 30 patients with IASD whose systolic PAP exceeded 60 mm Hg, oxygen also lowered the vascular resistance in the lung and this was followed by an increase in blood flow In three patients with PPH, however, Heath *et al* (60) observed no significant effect. Oxygen also did not alter the response of the pulmonary vessels to serotonin in the experiments conducted by Rudolph & Paul (101) with dogs

monary venous pressure), or vaso-occlusive (attributed to high pulmonary vascular resistance associated with anatomical lesions or functional vasoconstriction), has contributed to clarity of thought regarding this subject although combinations of several of these factors are involved in the genesis of most cases of pulmonary hypertension.

Support has increased for two important tenets: (a) That there is vasomotor activity in the lung; indeed, it appears that arteriospasm is often superimposed upon other mechanisms of hypertension in the pulmonary circulation. (b) Hypertension in the lesser circulation can, in itself, lead to a whole sequence of vascular changes; this inevitably evolves in a vicious cycle

Evidence of Vasomotor Activity in the Lung

De Burgh Daly (32), long a student of vasomotor activity in the lung, has written an excellent summary of much pertinent data Shepherd (106) has considered the subject with special reference to clinical observations in hypertension.

Effects of alveolar gas tensions—Borst and his co-workers (18), on the basis of a shift in the distribution of blood from a lung made to respire 10 per cent CO_2, established in the dog that there was a uniform and rapid vasoconstriction that reached its maximum at 2 to 3 min. When the O_2 tension in a lung was reduced, there was evidence of a homolateral pulmonary vasoconstriction at some time in the course of 10 of 18 experiments, but often not until after a series of ineffectual exposures to hypoxia. This time factor has not previously been appreciated The evidence of vasomotion in response to hypoxia seemed unaffected by the systemic arterial O_2 saturation by tetraethylammonium or hexamethonium given in doses sufficient to reduce systemic blood pressure, by bilateral vagotomy, or by administering enormous doses of pentobarbital sodium. Rodbard & Harasawa (96) found the pulmonary vasculature to be entirely passive to hypoxia. Like Borst *et al* (18), they measured pulmonary blood flow differentially by means of rotameters, but the cardiac output in their animals was low, and the possibility of damage to pulmonary nerve fibers was greater in their technique. Further study with direct measurement of pulmonary blood flow is indicated Aviado, Ling & Schmidt (6) concluded that their previous inability to confirm pulmonary vasoconstrictor effects of anoxia in dogs might have been the result of damage to nerve fibers during cannulation of lobar pulmonary vessels They repeated their experiments with avoidance of this difficulty and found that inhalation of 5 to 10 per cent O_2 could result in either a decreased or augmented pulmonary vascular resistance, since at least four opposing factors were operative. Reflex pulmonary vasoconstriction could be demonstrated only if the perivascular sympathetic fibers were intact, and was considered to result from anoxic stimulation of chemoreceptors in the carotid and aortic bodies Local pulmonary vasodilatation was, however,

vasoconstrictor action on the pulmonary vessels than any other known drug or neural reflex. In dogs under a constant pulmonary infusion of this drug, there was an increase in pulmonary vascular resistance and also a 60 per cent increase occurred in cardiac output, and this could not be reversed by high alveolar oxygen tensions, although it was counteracted by certain drugs It is noteworthy that systemic arterial pressure fell while 5-HT was being infused There was no effect when the 5-HT was injected into the atrium. Borst *et al.* (17) also found 5-HT to be markedly vasoconstrictor for the vessels of the lung.

On the basis of anatomical evidence involving the "gnarling" of casts of the vessels, Patel & Burton (92) concluded that both norepinephrine and naphazoline hydrochloride (Privine) produce venous as well as arterial constriction. Functional evidence for the pulmonary vasoconstrictor action of the former and of epinephrine was obtained by Borst and co-workers (17).

Reflex activity involving the pulmonary vessels.—It was demonstrated by Downing (36) that pressoreceptors appropriately stimulated in the pulmonary vessels of the dog can initiate components of the Jarisch-Bezold reflex. The pressure was raised throughout the vasculature of the left lung by appropriate cannulation of the left pulmonary artery, and temporary occlusion of the draining veins Reflex pathways were shown to involve the homolateral vagus, but the anatomical location of the sensitive tissue could not be established. The pressure in the general pulmonary circulation remained unaltered in these experiments. Sanger and co-workers (102), however, showed that upon raising the venous pressure within one lung to between 35 and 45 mm. Hg by cannulation and infusion of saline into the veins, the general PAP and vascular resistance rose considerably. This did not occur when the lung had been previously denervated. The location of the pressoreceptors again was not established in this study. The experiments of both Downing and Sanger were done in animals with closed chest.

In patients with MS several observations suggest the existence of vascular tone which Wood (120) has called "reactive hypertension." According to Semler *et al.* (105), after valvulotomy the mean PAP fell proportionately more than the mean wedge pressure; on the contrary, upon exercise, the mean pulmonary arterial pressure rose proportionately more than the latter.

The radiographic observations made by Simon (110) suggest that a critical rise in pulmonary venous pressure can initiate a localized vasoconstrictor reflex. This conclusion is based on the smaller size of the lower lobe pulmonary veins in patients with MS where the pressure was highest, while the upper lobe veins appeared to be engorged. This engorgement was greatest when the pressure approached 27 to 28 mm Hg, but it was less with pressures higher than this. Further confirmation of such evidence of regional vasomotor activity would be of great interest

Ferguson & Berkas (46) found that when the left upper lobe artery was

Effects of drugs.—Reports on the vasodilator action of acetylcholine have been further investigated. Fritts *et al.* (50) found a fall in PAP without change in "wedge pressure," was much more evident after the induction of hypoxia and concomitant pulmonary hypertension. Wood *et al.* (124) observed a transient decrease in pulmonary vascular resistance in patients with MS treated with this drug, and there was a maximal decrease in PPH, but patients with the "Eisenmenger syndrome" proved refractory (120). All of the patients in the group of 13 under study with this condition were over five years of age, and the failure of response may have been associated with the presence of obliterative disease in the vessels, or with a special property of vessels of "fetal type." In the experiments of Rudolph *et al.* (100), acetylcholine as well as histamine and adenosine triphosphate markedly reduced pulmonary vascular resistance which had been elevated by continous infusion of 5-HT, although in normal dogs these drugs were found to produce a slight increase in PAP and cardiac output. Borst and co-workers (17), in view of the fact that the use of the Fick principle requires a constant state not easily attainable in the animals under the influence of rapidly acting drugs, substituted a pump for the right ventricle and estimated the effects of various substances injected into one lung by changes relative to the other lung in blood flow measured directly by means of rotameters. They found that acetylcholine increased transpulmonary pressure as a result of an augmented bronchomotor tone, but that when this effect was prevented by permitting the lungs to collapse, the vasomotor effects in these organs were inconstant and small. Aminophylline produced pulmonary vasodilatation but only in some animals The conclusion reached by Quimby, Aviado & Schmidt (93) regarding this substance was the same, but they also stated that it increased the force of myocardial contraction. They tested numerous other xanthines, some of which affected the pulmonary vessels in a manner similar to aminophylline, whereas others were vasoconstrictor, but in every instance the vessels of the extremities reacted like those in the lung.

Tolazoline hydrochloride (Priscoline) in a patient with PPH was found to reduce PAP and pulmonary arterial resistance, with some increase in cardiac index Hexamethonium, however, in two patients with the same disease did not induce a fall of the pulmonary vascular resistance, but the PAP and blood flow both declined (30) In MS Balchum, Gensini & Blount (8) observed that, at rest, hexamethonium produced a fall in pulmonary arteriolar resistance, but that the latter rose sharply with exercise, although not to the same extent as when the drug was not given. In the experience of Yu and co-workers (127), however, this drug, similarly introduced via cardiac catheter in patients with the same disease, only occasionally diminished the pulmonary vascular resistance While the mean PAP declined significantly in over half of the subjects there was usually no change in the gradient to the LAP.

Rudolph & Paul (101) concluded that serotonin has a more powerful

Heath *et al.* (60, 66) have made the observation that in IASD the grade of the vascular lesion is not particularly related to age, but is closely related to the level of the mean PAP. With large PDA or IVSD where, in contrast, hypertension in the lesser circulation exists from birth, the higher grades of lesions were usually found in the older patients Again, the PAP and pulmonary vascular resistance were closely correlated with the anatomical grade. In 32 patients chiefly with IVSD, it was found that if the lesions were of "grade 4" or higher, hypertension in the lesser circulation could not be reduced by repairing the defect In their opinion, lesser grades could be considered as implying "high resistance, high reserve." This is further evidence for the view that a high blood flow does not in itself result in lesions in the pulmonary vessels (63, 64).

Short (109) has emphasized that a small artery with a thick muscular wall may represent the appearance, when in spasm, of a larger vessel with a normally thinner wall Anyone who has observed the intestine in various states can appreciate the significance of this point. It is clear that some independent level of reference, such as a relationship to some order of branching of the bronchi, is needed before measurements of vessels and their component coats can be evaluated in terms of hypertrophy and hyperplasia rather than "contracture."

Atheromatous changes—Heath and his co-workers (66) have established that the major pulmonary arteries showed no atheromatous changes in conditions in which the pulmonary arterial pressure and blood flow are diminished, but that when pulmonary hypertension existed from birth [illegible]

monary arteriosclerosis produced by embolization in rabbits, Heptinstall (67) found that an induced hypercholesteremia resulted in an increased amount of cholesterol within the arterial lesions. Thomas *et al.* (118) observed that corn oil, an unsaturated fat, had no such effect, in contrast to their previous experience with the saturated fats of butter and oleomargarine.

The elastic coat—It is noteworthy that in the giraffe, an animal in which the mean pulmonary arterial pressure is normally in the range of 80 mm of Hg, there is more elastica and less of the phylogenetically older muscle. The pulmonary artery in this species can be as much as 1 cm thick [Goetz & Meyer (53)] Heath & Best (57) have written that when venous hypertension is present from birth, the pulmonary artery has "fetal" characteristics with more elastic tissue and relatively little muscle like the aorta

An increase with age has been found by Meyer & Richter (90) in the total mass of the trunk of the pulmonary artery and its two major branches (the "Pulmonalisgabel"); in pulmonary hypertension there is a striking rise

connected to a systemic artery, pulmonary edema developed after denervation at the same or at a lower arterial pressure than that tolerated before denervation.

Vascular Changes in Pulmonary Hypertension

General considerations.—Barnard (10) has stressed the necessity of distending the vessels for histological studies to avoid artifacts that he ascribed to post-mortem collapse or contraction.

Heath & Best (57) have pointed out that the lingula normally contains arterioles of less than 100 μ diameter that have a distinct muscular media, and that it is therefore an undesirable source of biopsy tissue in attempting to determine the presence of lesions, despite its surgical convenience. Landtman & Hjelt (77) and also Bor & Valach (16) used the lingula for biopsy *in patients with PDA and this may account for the high proportion that* were considered to have vascular changes. It is also necessary to realize, as has been stressed by at least two groups of observers (15, 107), that vascular lesions in pulmonary hypertension may be focal.

Staemmler's idea that hypertension in itself can damage pulmonary vessels has been abundantly confirmed. The correlated anatomical and functional data obtained by Heath and his associates (59 to 61) represent an important contribution Heath & Edwards (59) divided the changes in the small *pulmonary vessels observed in patients with pulmonary hypertension* associated chiefly with congenital septal defects of the heart into six grades: Grade 1—Retention of pulmonary vessels with fetal structure (it is necessary to note that in acquired hypertension arterioles much less than 100 μ in diameter may also have a distinct muscular coat). Grade 2—Medial hypertrophy with cellular proliferation in the intima Grade 3—Progressive fibrous vascular occlusion by intimal fibrosis in arteries of approximately 300 μ diameter In addition, some vessels may show the characteristics of *grade 4. Grade 4—Abundant "dilatation lesions" These changes involve* dilatation of vessels proximal to occlusion, or beyond occlusive lesions Dilatation also involves small muscular pulmonary arterioles that become filled with proliferated tissue of plexiform structure often associated with thrombosis Additional thin-walled vessels may be associated with these plexiform lesions, or seem to accompany or to branch off beyond vessels occluded by plexiform masses The nature of these "angiomatoid lesions" is still under study. Grade 5—Numerous dilatation lesions, some in a state of organization; medial as well as intimal fibrosis Pulmonary hemosiderosis, possibly associated with bleeding from thin-walled veinlike branches of muscular arteries Grade 6—Necrotizing arteritis, which is said to be rare but which has been reported in various types of pulmonary hypertension of both "primary" and secondary types These criteria are not entirely sharp Furthermore, the pathogenesis of the various changes that are, perhaps unjustifiably, grouped together as "dilatation lesions" may be variable Nevertheless,

the latter condition studied angiographically by Evans, Short & Bedford (42), prolific anastomoses between the two circulations were demonstrated, although the extrapulmonary bronchial arteries were not thought to be enlarged. In another patient with PPH who died at the age of five weeks and in whom pulmonary arterial changes consisted simply of a striking hypertrophy of muscle without angiomatoid lesions, the bronchial arteries were not enlarged, but in others with pulmonary hypertension accompanying large septal defects, both the intra- and extrapulmonary bronchial arteries were enlarged and the former communicated freely with the pulmonary arteries (79). Thus, there is no evidence that enlargement of the bronchial arteries precedes the development of primary hypertension, but rather that their expansion is associated with the appearance of obstructive lesions. Again, it is not surprising that newly formed granulation tissue in the lung should be supplied from the systemic circulation. This would account for the penetration of the walls of vessels the lumina of which are filled with plexiform masses. The remarkably thin walls of vessels composing the angiomatoid lesions do not deny their source in the systemic arterial circulation, since similar vessels have been observed in an experimentally induced pulmonary collateral circulation Further observations are necessary in order to clarify the entire subject of the angiomatoid lesion and of its vascular connections.

Angiographic studies.—Angiographic studies in autopsy material (40 to 42) have demonstrated evidence of loss of vessels associated with various obstructive lesions which gives a "pruned" appearance. Less attention has been paid to the tortuosity evident in the published angiograms (109) which is demonstrable also in plastic casts (79). It is possible that some of the tortuous vessels may belong to the collateral circulation. Similar angiographic investigation in MS made by Doyle *et al* (37) revealed enlargement of the main arteries and narrowing of the smaller branches confined to the lower and mid-zones of the lung, while in congenital heart disease with bidirectional shunts the peripheral vessels often were small in all lung fields. In other lungs from congenital heart disease, the enlargement often extended into the small branches. Heath & Best (57) found that in MS the thickness of the medial coat was greatest in vessels in the lower lobe, while this change tended to be more uniformly distributed in both upper and lower lobes in congenital heart disease with pulmonary hypertension.

Attempts to Produce Experimental Vascular Lesions in the Lung

The sequence of events following anastomosis of the distal left pulmonary artery to the subclavian artery in dogs has been traced by Dammann *et al.* (33). If the mean PAP exceeds 35 mm. Hg at once the animal is likely to die of pulmonary edema, but if the pressure is less than 30 mm. Hg

that correlates with the weight of the right cardiac chamber. The increase at first is predominantly muscular, then elastic, with ultimate loss of muscle.

Other medial changes.—Extensive discussions regarding the degree and distribution of muscular thickening in various types of pulmonary hypertension have been published by Heath and his co-workers (57, 63, 64). The idea persists that defects in the media of the pulmonary arteries represent congenital focal hypoplasia or aplasia of the media, whereupon they serve as the basis for fibrosis and other changes in the pulmonary vessels (41, 42), but this concept is held in question by many who point to the frequency of the lesion in acquired pulmonary hypertension (117). Others have explained it on the basis of "arteritis" in the broad sense that would include the possibility of mechanical factors in the pathogenesis (34).

Three of the ten patients with PPH in the series presented by Wade & Ball (119) had "pulmonary arteritis," and similar lesions were described by Heath, Whitaker & Brown (65) in this disease An anatomically similar lesion was described by Johnstone & Smith (70) in another instance of chronic severe MS without acute carditis or arthritis, and another by Rose & Spencer (97) who also observed a rare example in association with severe pulmonary emphysema. Rose & Spencer (97) commented that one distinction from periarteritis nodosa as it involves the pulmonary vessels is that the latter tends to be more often granulomatous.

Forbes (49) has described fatal hemoptysis in a patient with severe fibrinoid necrosis and "arteritis" of the pulmonary arteries. Cystic medionecrosis with incomplete rupture of the main pulmonary artery has been found in PPH (94), as well as in hypertension associated with the common ventricle (79).

Plexiform and angiomatoid lesions.—The angiomatoid lesions have been described particularly in patients with major intracardiac or aortopulmonary shunts (22, 41, 54, 63, 98), in PPH (34, 42, 68, 76, 83, 119), and in a definitely acquired condition, pulmonary schistosomiasis (52, 79). The idea that these lesions represent nothing more than an organizing blood clot has been generally rejected (98), but the occurrence of thrombosis in the smaller channels cannot be denied. "Sequestration" of platelets beyond the occlusion has been described in several instances as a process that can contribute further both to obstruction and to the accummulation of granulation tissue in the plexiform lesions (79) Arteriovenous communications have been sought in serial section (22, 98), and it has been established that a few of the lesions drain into the pulmonary veins. The angiomatoid lesions do not, however, regularly represent arteriovenous communications nor is there any good evidence that they are congenital malformations On the contrary, it

(15, 25, 27, 28, 68, 79). Carpenter & Prichard (25) had favored the idea of the persistence of the fetal state of arterioles less than 100 μ in diameter. Necrotizing, periarteritis nodosa-like, angiomatoid, thrombotic, and other lesions in their acute or healing stages are quite similar to those encountered in hypertension secondary to large cardiac septal defects and the like, and can be interpreted as being primarily the result of the hypertension (65, 79). Once they are in progress, however, they contribute to a vicious cycle (Fig. 1).

Of interest, but of unknown significance are features of the natural history of the disease, as the predominance in females (30, 34, 42, 76, 125) and

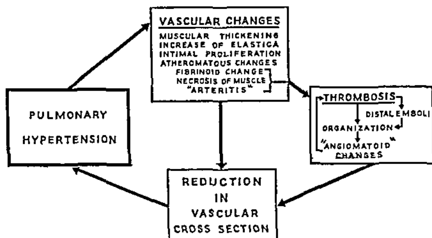


FIG 1 Vicious cycles existing in the pulmonary circulation under hypertension of sufficient degree and duration (79)

the fact that most patients are between 20 and 40 years of age. In some, a relationship to pregnancy has been questioned with particular reference to the possibility of amniotic fluid or other embolism in that state, but no actual evidence has been provided. The occurrence of the disease in three siblings has been reported by Coleman *et al.* (29); the significance of the fact that all had had diphtheria as young adults is unknown.

Clinical features—Heath, Whitaker & Brown (65) have noted that chest pain similar to
nitroglycerine. Hemop
with PPH [4 per cen
It was found in 33 per cent of Wood's (121) patients with the "Eisenmenger's syndrome" and was the important mechanism of death in 29 per cent of 42 fatalities. Desaturation of the systemic arterial blood has been noted in 50 per cent or more of patients with this disease in the terminal

pulmonary arteriosclerosis does not develop. With a shunt involving only the left upper lobe artery, survival with the development of changes in the vessels is more likely After initial hemorrhage, medial hypertrophy may be evident as early as three weeks, with centrifugal spread to involve most of the vessels by eight weeks. There is also intimal fibrosis and cellular proliferation in the smaller vessels.

To test the hypothesis that acute pulmonary hypertension can damage pulmonary vessels, Brenneman & Liebow (21) injected sufficient saline solution or blood into the main artery of a left lung whose veins were temporarily occluded to raise the pressure in an oscillating fashion to levels as high as 150 mm. of Hg over short intervals of time. Except for thrombosis of occasional vessels, no vascular lesions were observed. Several days later, the lungs recovered remarkably well The observed absence of vascular lesions was thought to be because the hypertension was of insufficient duration, or because spasm of arterioles did not occur as a result of interference with nervous pathways, or because a preliminary phase of muscular hypertrophy in the pulmonary arterioles might be necessary.

After long-continued injection of rabbits with serotonin, Rossi & Zamboni (99) found adherent organizing thrombi in the large pulmonary arteries, and varying degrees of intimal fibrosis and elastic and endothelial hyperplasia in the smaller arteries and arterioles The pulmonary veins were not affected.

Remarkable muscular hyperplasia with stenosis of pulmonary arteries was produced by Kell *et al* (71) in cats by means of repeated injections of pentylenetetrazol (Metrazol) or by electrical cerebral stimulation.

Falkenbach *et al* (44) have reported that surgical constriction of one major pulmonary artery in puppies results in a great rise in the general PAP. Their suggestion that this is analogous to the Goldblatt phenomenon is startling and requires unequivocal experimental confirmation.

Primary Pulmonary Hypertension

Pathology and natural history.—What is now generally called primary pulmonary hypertension continues to be puzzling, although a considerable number of patients have been diligently investigated Earlier ideas that this condition was the result of arteriolar sclerosis or the consequence of more or less inapparent embolism which must indeed always be considered, are yielding to the concept that it may be initiated by a spastic state of the pulmonary vessels In part, this is based on the reduction in pulmonary vascular resistance achieved by the use of pharmacological agents, by the demonstration of an element of "reactive hypertension" in MS and other conditions, and by the observation that severe PPH can exist with little or no anatomical change in the pulmonary vessels (42). The earliest arterial lesions, as judged by the study of lungs of young children with primary pulmonary hypertension, is thickening of the muscular coat of small arteries

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the level of pulmonary arterial hypertension at rest or under mild exercise seems to be directly related to the "functional incapacity" of patients who had undergone resection of pulmonary tissue.

Griffin *et al.* (55) commented on the rarity of cor pulmonale in children with asthma, but that it is to be considered as a complication when response to treatment is poor.

Littlefield *et al.* (84) have demonstrated that in the presence of an expanded bronchial collateral circulation, extreme overdistention of the pulmonary vessels and hemorrhage can occur during cardiopulmonary bypass under elective cardiac arrest with simultaneous aortic and pulmonary artery occlusion. Thus, in analogous clinical circumstances, decompression of the left atrium is imperative.

In a patient with aldosteronism Ferri *et al.* (47) obtained essentially normally hemodynamic data.

Newer Methods in Diagnosis and Treatment

In the assessment of anticipated effects of surgery in PDA, Actis-Dato & Tarquini (1) reported a method for measuring pulmonary artery pressure while the ductus was occluded by means of a balloon on another catheter introduced from the aortic side. A rise of PAP occurring at once or on slight effort was thought to indicate an ominous prognosis.

Swan *et al.* (115) observed that those patients with IASD in whom there was a decline in resistance when oxygen rather than air was respired, had a relatively high operative survival rate This may prove to be a useful test under similar circumstances of disturbed hemodynamics [see also Shepherd (106)]

To provide a simple indication of the direction of shunts, Kudasz (75) provided the cardiac catheter with a freely moveable pliable radio-opaque flap.

In the treatment of patients with IVSD, Therkelsen *et al* (116) found useful the method devised by Dammann & Muller in which a stenosing band is placed about a major pulmonary artery. Dyspnea was observed to decrease in survivors of this operation which, in their hands, carried a mortality in excess of 50 per cent.

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CARDIOVASCULAR DISEASE: PERINATAL CIRCULATION[1]

By Leo Stern, M.D.[2] and John Lind, M.D.
The Department of Pediatrics, Karolinska Hospital, and Wenner-Gren Research Laboratory, Norrfull's Hospital, Stockholm, Sweden

*With the process of birth, the human infant is transformed from a para-*sitic foetus to an organism which must be capable of independent biologic function. Along with this transformation changes occur in the newborn's cardiovascular system to adapt him to his new role. The infant's survival is dependent to a large extent upon the smooth and successful accomplishment of these changes.

The Foetus

Since Harvey's description in 1628 (1), the foetal circulation has been the subject of much discussion and speculation Largely on the basis of anatomical examination, the concept had evolved that venous blood from the foetal head, returning via the superior vena cava to the right atrium, entered the right ventricle and proceeded mainly via the ductus arteriosus to the descending aorta to reach the placenta. On the other hand, the comparatively well-oxygenated blood in the inferior vena cava, coming from the placenta through the umbilical veins, bypassed the liver via the ductus venosus, was shunted through the foramen ovale into the left atrium, and went, for the most part, by means of the left ventricle and ascending aorta to the head of the foetus. This proposition had the attractive advantage of directing the most highly oxygenated blood to the brain and coronary arteries, and the less oxygenated blood to the placenta, there to be oxygenated; a most efficient arrangement, indeed

The difficulty lay mainly in the fact that such an arrangement appeared to suppose a crossing of two independent streams in the right atrium Almost two hundred years ago, in 1774, Sabatier (2) proposed that the two caval streams did not mix He reasoned that the foramen ovale was placed not between the two atria but between the union of the two venae cavae and the left atrium Two years later, in a paper read in St Petersburg, Wolff (3) amplified this further. He reasoned that the foetal atria were not in communication with one another but that the inferior vena cava was interposed between them and had independent openings into each of them In 1826, Kilian (4) proposed the division of the stream of the inferior vena cava into two parts He supposed that the left portion passes unmixed via the foramen ovale to the left atrium, the right portion mixing in the right atrium with superior vena caval blood.

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[2] Medical Research Fellow, National Research Council of Canada

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Tying the cord in the lamb causes an immediate small rise in blood pressure [Dawes (21)]. The explanation offered is that tying the cord stops the large flow of blood through the umbilical arteries and thus increases the systemic arterial resistance. This finding has, however, not been verified in man (22, 23), and recent work by Lind *et al* (24) suggests that neither tying the umbilical cord, nor the initiation of respiration results in any immediate increase in pressure in the cord. It should be pointed out that the difference in results in lamb and human can be accounted for not only in terms of species difference, but also by the fact that the ratio of the umbilical artery pressure to the venous pressure, is much higher in the lamb than in the human subject (6), conceivably resulting in a different set of haemodynamic responses. Ashworth & Nelligan (25) have recently reported changes in human neonatal blood pressure occurring within the first few hours of life, manifested as a fall to a level often as low as one-half the birth value.

The question of early versus delayed clamping of the umbilical cord has been the subject of much controversy. As 10 to 15 per cent of the circulating blood volume is trapped in the placenta, holding the baby at a level below the mother, stripping the cord and waiting till all pulsations cease will result in an increase in the amount of blood transferred to the infant (26, 27). DeMarsh *et al.* (28), by means of dye studies, demonstrated an increase in blood volume in infants where clamping was delayed. They also observed increased reticulocyte counts in the first few days of life in those with cords clamped early (29), and suggested this to be "a compensatory result of the deprivation of their full complement of blood" It has also been suggested that early clamping will reduce the iron stores transferred to the infant, thereby tending to promote an iron deficiency anaemia (30). On the other hand, it is questionable whether these advantages outweigh the potential effects of overloading the circulation, especially in an infant with cardiorespiratory distress Particularly would this be so in the case of the premature, for the younger an infant the greater is the proportion of blood in the placenta As Dawes has put it, "there is no reason at all why plethora should be not just as dangerous for the newborn infant as for the adult" (21).

The Ductus Venosus and the Hepatic Blood Flow

The precise function of the ductus venosus is not certain Not all species of mammals possess one at maturity Thus, it disappears at an early stage of gestation in the horse (31) and pig (32) Experiments have shown that occlusion of the ductus venosus in mature foetal lambs causes no significant change in blood pressure, heart rate, or carotid arterial O_2 saturation [Amoroso *et al.* (33)]. The authors conclude that the ductus venosus is not essential to the foetal lamb In the human foetus, the umbilical vein sends branches to the left lobe of the liver, whereas the right side of the liver

In 1927, Huggett demonstrated that it was possible to deliver a foetus still attached to the mother with the umbilical cord intact and in reasonably good physiological condition, thus initiating a new era of experimental foetal physiology. He found the oxygen saturation of carotid arterial blood in the goat to be higher than that in the umbilical artery (76). In recent years, Barclay, Franklin & Pritchard (6), following the pioneering work of Sir Joseph Barcroft (5) showed, in a series of cineroentgen experiments in lambs, the separation of the blood in the inferior vena cava into two streams, and the superior vena caval stream flowing into the right ventricle, the ductus, and the aorta. Windle (7) confirmed these findings in the cat and guinea pig by injecting India ink and observing the heart and great vessels directly through a thoracic incision. The possibility of studying human foetal circulation by means of angiocardiography was first demonstrated by Lind & Wegelius (8), who then went on to show visually the details of the foetal and neonatal circulation, confirming the functional relationship between the inferior vena cava and the left atrium, and demonstrating the changes occurring in the transition of the one to the other (9, 10).

With the aforementioned background it is now proposed to analyze in some detail the changes in the component parts of the circulatory system occurring as a result of the change from foetal to neonatal life, and some clinical implications of their normal and abnormal patterns.

The Umbilical Cord

At birth, the umbilical cord contains 3 vessels: the right and left umbilical arteries and a single vein (the left one). The postnatal changes in the vein have recently been described by Butler (11). Functionally, it would appear that tying the vein in the lamb results in a diminution of venous return and thereby a lowered pressure in the right atrium, which may assist in the closure of the foramen ovale [Dawes *et al* (12, 13)]. The umbilical arteries close by virtue of contractile muscle in their walls It would appear that the stimulus for closure is the oxygen tension of the blood in the arteries. Thus, high O_2 will constrict the vessels and low O_2 and high CO_2 contents will dilate them (14). Perfusion experiments have amply supported this view (15, 16, 17), suggesting that the postnatal rise in blood oxygen with the onset of respiration is the prime cause of arterial closure. Pulsation in the cord normally dies down not long after birth, the average time for the process being about 10 min (18) The observation that in cases where respiration is delayed the cord continues to pulsate is not a new one, having been made by Bondi in 1905 (19). Recently, Desmond and his co-workers (20) have shown that continued and prolonged pulsation of the cord appears to be associated with "difficulties in the transition from intrauterine to extrauterine life." They suggest that this is caused by the decreased contractility of the musculature brought about by anoxia or asphyxia, or both.

changes to effect functional closure. Lind & Wegelius (9) were able to inject the umbilical vein of a newborn infant with contrast medium before and immediately after the first breath. By means of serial films they demonstrated the functional closure of the foramen ovale with the onset of respiration. A possible application of this exists in the giving of gastric oxygen or oxygenated blood via the umbilical vein in cases of asphyxia neonatorum. As the foramen ovale is open prior to respiration, the oxygen or oxygenated blood administered will go via the foramen ovale directly to the brain and medulla, the organs most likely to be damaged permanently by prolonged oxygen lack.

It is important to point out that while functional closure ensues almost immediately after birth, anatomical closure is not effected for some weeks after (39, 40). Thus, the potential exists for a reopening of the foramen ovale in response to changes in pressure on either side if it, capable of reversing the normal left to right pressure gradient. That this is so is suggested by the work of Prec & Cassels (41) who, using an ear oximeter in infants less than 4 days of age, found that in response to crying (increased pressure in the right atrium) the O_2 saturation of the blood fell even on breathing artificially high O_2 mixtures. They concluded this to be attributable to a right-to-left shunt at the atrial level. Lind & Wegelius (9) examined 30 infants angiocardiographically for persistent or recurrent cyanosis in the first week of life. All showed a right-to-left interatrial shunt decreasing in magnitude with time. The significance of this latter finding will be discussed in a later section.

The Ductus Arteriosus

In foetal life the ductus arteriosus acts as a bypass for blood around the non-functioning lungs. Since the pressure in the pulmonary artery is higher than that in the arota, the flow is from right to left, i.e., pulmonary artery to aorta. If this channel fails to close after birth, when the pressure relationships in the aorta and the pulmonary artery are reversed, the flow will be from left to right (aorta to pulmonary artery). The question of the stimulus to normal ductus closure is a vexing one and by no means certain. The mechanism and timing of the closure has occasioned far more controversy than has the closure of any other foetal channel. The widely held view at present is that closure of the ductus takes place after birth, the first stage consisting of functional closure followed by anatomical obliteration. This view has been recently challenged by Sciacca & Condorelli (68), who propose that closure is actually begun during foetal life. Their work, based on morphologic and experimental investigations in guinea pigs, has led them to conclude that "involution of the ductus is, in itself, an intrinsic phenomenon, which is entirely independent of any functional changes affecting it at birth."

In recent years attention has been focused upon the role played by oxy-

is supplied by the portal system, which has a lower O_2 saturation in the foetus Recent reports have appeared of a number of newborn and stillborn infants with degenerative changes in the liver more severe on the right side than on the left (34, 35). In addition, Emery (36) has found the distribution of hematopoietic foci in the infantile liver to be greater in the right lobe than in the left. These differences may be caused by a variance in O_2 supply to the two lobes With the interruption of the umbilical cord, the major portion of the hepatic blood flow changes from the highly oxygenated umbilical vein blood to portal venous blood with its relatively low O_2 content. The possibility exists that this might in some measure account for the development of functional liver disturbances in the newborn period (10).

The ductus venosus in man has an innervation from the vagus and possesses a sphincter at its distal end It has been postulated that this closes when the venous return is high in the umbilical vein (i.e., during a uterine contraction) to divert the liver blood in high volume, in order to prevent sudden overloading of the foetal heart [Reynolds (27)] Lind and Wegelius, using rapid film angiography, have shown the course of the flow through the ductus in the foetus (8) and its functional closure immediately after birth (9) To quote Sir Joseph Barcroft (5). "It is perhaps less important that the ductus venosus should be patent during foetal life rather than it should be closed during adult life." The precise nature of the stimulus to closure is uncertain Both neurogenic stimuli via the vagus and the sudden lowering of pressure in the umbilical vein have been suggested It is possible that in situations of neonatal distress its patency would provide a rationale for the administration of gastric oxygen or oxygenated blood via the umbilical vein, as otherwise most of the extra oxygen would go to the liver On the other hand, the continued patency of the ductus venosus, in a situation where the heart is already under stress, would, by causing a rapid shunting of blood almost directly into the right atrium, throw a further strain on the infant's struggling cardiovascular system

The Foramen Ovale

Functional closure of the foramen ovale occurs almost immediately after birth Barcroft (5), by means of a photoelectric cell tied around the carotid artery of a lamb foetus, showed the blood to be 100 per cent oxygenated by 4 min after ligation of the umbilical cord (possible only if there is no right-to-left flow through the foramen ovale) Dawes and his co-workers (37) studied the pressure changes in the great veins and left atrium in the lamb They showed that with the onset of respiration the pressure in the left atrium rises because of the tremendous increase in pulmonary flow This, coupled with the fall in pressure in the inferior vena cava (and hence in the right atrium) caused by the diminished venous return resulting from interruption of the placental circulation, provides the necessary pressure

The evidence for postnatal patency of the ductus in the human infant is more varied Eldridge *et al.* (51) found a difference in O_2 saturation of as much as 10 per cent between the arms and legs of infants up to 3 days of age. This would indicate a right-to-left shunt. But the taking of blood could well result in crying sufficient to raise right sided heart pressure and reverse the direction of a left-to-right shunt. Prec & Cassels (52) found dye dilution curves suggestive of bidirectional shunts at the ductus level in infants less than 15 hr. old. Rowe & James (53), using mongols, and Adams & Lind (49), using normal newborn infants, were able to find on the basis of O_2 saturation studies at catheterization the presence of left-to-right shunts at the ductus level up to two weeks of age Burnard (54, 55) has recently reported the existence of a murmur presumed to arise from the ductus arteriosus in a large proportion of newborns He noted it to be commoner in prematures, in asphyxial states, and in cases where the normal neonatal temperature drop had been prevented (56). In our laboratory it has been possible to demonstrate angiocardiographically the presence of ductal patency in the neonatal period, a finding also reported recently by James (58).

The possible advantage of such a patent ductus in the newborn baby is questionable. Unlike the lamb whose lungs are normally not fully expanded for about one hour after birth, the human infant has been shown radiographically to expand his lungs almost completely with the first good cry. The entire event occurs within a period of a few seconds or less (57). Thus, the advantage proposed for the lamb seems not to apply in the normal newborn infant but might be of importance in a distressed infant. A shunt of large magnitude could precipitate heart failure It is, however, also possible that a small left-to-right shunt will benefit the infant to the extent that the increased flow to the left atrium will raise the pressure in that chamber and contribute to the functional closure of the foramen ovale

Changes in the Lungs and Pulmonary Artery

In the foetus the lungs are not aerated, the pulmonary resistance is high, and the pulmonary blood flow reduced; oxygenation and CO_2 exchange are carried out by the placenta Dawes *et al.* have determined the pulmonary flow in the foetal lamb to be about 12 per cent of the total cardiac output, calculating the values from the measurement of oxygen content (21, 59) Studies of the human foetal circulation up to midterm (Lind & Wegelius) suggest a similar proportion on the basis of the relative distribution of contrast media during angiocardiographic study (9). With the onset of extrauterine life the lungs are aerated and expand. This event acts as a focal point around which many of the changes in the perinatal circulation occur

The mechanics of lung expansion have been the subject of considerable discussion. Jaykkä (60, 61) has proposed this to be a result of capillary erection arising from the increase in flow in the pulmonary artery He was able to bring about expansion of foetal alveoli by injecting liquid (Macro-

genation of the blood in closure of the ductus arteriosus. In a retrospective study, Record & McKeown (42) obtained evidence of an incidence of foetal distress at birth which was substantially higher in cases of patent ductus arteriosus than would be expected among normal births. Using guinea pigs, they found a wider patent diameter of the ductus in those animals subjected to a reduced O_2 content in the inspired air for 24 hr. after birth than in controls (43). This latter work complements that of Kennedy & Clark (44), who showed that closure of the ductus in the guinea pig could be effected by inspired oxygen independent of any nerve supply to the ductus. Born *et al.* (45), using newborn lambs, *noted ductus constriction in response to inflation of the lungs with oxygen.* Not only did the ductus tend to close with O_2 administration, but it assumed the anatomical position it takes in 1 to 2-day-old lambs (angle between the ductus and the aorta becomes more acute). There was no constriction in response to inflation of the lungs with nitrogen. The effect was seen even after sympathectomy and destruction of the brain and spinal cord, implying a direct effect of O_2 on the ductus. They also noted that in addition to O_2 as a stimulus to constriction, extreme asphyxia, *somewhat paradoxically, causes the ductus to constrict, presumably via the* release of sympathetic amines. This effect was reproducible by the administration of epinephrine and norepinephrine Reynolds (46) has postulated that ductus closure occurs in response to pressure changes at either end of it However, Dawes and his co-workers point out that the constriction occurs first before the pressure changes, and against sufficient intravascular flow to produce a murmur (47).

Until recently, it had been supposed that functional closure of the ductus occurred shortly after birth. Anatomical studies indicate that at the end of the first week of life calibre patency of the ductus averages 2 mm. (48). Dawes *et al.* (47), however, have shown that in the newborn lamb the flow in a ductus opening that will admit only a pinhead, amounts to 40 ml /min It is thus apparent that a shunt even through a minute opening could be of clinical importance. Recent studies in both lambs and man suggest that the ductus does, in fact, remain at least partially open for some time after birth. Dawes and his co-workers (50) were able to demonstrate by cineangiography, patency of the ductus for a period of some hours after birth. In addition, they were able to record a murmur and thrill characteristic of a patent ductus arteriosus from the ductus directly (47) They calculated *that the volume of blood flow through the ductus is at first very large,* amounting to as much as half the total pulmonary flow one-half hour after birth. They reasoned that during the first hour of life when the lungs are not fully expanded, patency of the ductus may serve a useful purpose as it *enables partly oxygenated blood to recirculate through* the lungs and thereby pick up more oxygen from those portions of the lung which are adequately ventilated. Their demonstration that temporary occlusion of the ductus caused a fall in carotid arterial O_2 saturation, reversible on removing the occluding tapes, would appear to support this view.

that although the calculated pulmonary vascular resistance within one hour after delivery is relatively high compared to that of the older infant or adult, it is still considerably less than the systemic vascular resistance and represents a drop to almost one-twentieth of the calculated foetal value. Histological studies have shown that the foetal structure of the small pulmonary arterioles, having a high ratio of muscular wall thickness to lumen size, persists for the first few months of life (66, 67). The further decline in pulmonary vascular resistance correlates well with the decrease in media thickness and subsequent increase in the width of the lumen of these vessels In addition, it is possible that the continued patency of the ductus arteriosus, by increasing the flow, tends to keep the pressure in the pulmonary artery at higher levels In this connection it is interesting to note in the catheter studies of both Rowe & James (53) and Adams & Lind (49), that the approach to "normal" levels of the pulmonary arterial pressures coincided approximately with the disappearance of the previously demonstrated left-to-right shunts at the ductus level.

Changes in Heart Size

Roentgen studies [Lind & Wegelius (10)] showed that if the cord is clamped before the first breath, a striking immediate diminution of the heart size occurs in the first 3 to 4 systoles, followed by a corresponding cyclic increase in size. This transient decrease in heart size can be explained by the opening of the pulmonary vascular bed at the same time that the venous return through the umbilical vein is cut off. When the flood of blood has passed the lung vessels, which takes 3 to 6 heart cycles, the heart fills again with new blood and a haemodynamic equilibrium of inflow and outflow is established

Studies of the heart volume in normal newborn infants [Kjellberg *et al.*, (69)] show that there is a significant reduction, averaging 25 per cent of the initial value, in heart volume on the second day of life. The change is not related to any change in muscle mass and appears to be caused by the decrease in blood volume as a result of the cessation of the placental circulation Another factor contributing to this diminished volume is the haemoconcentration which occurs in the first days of life (69) In addition, it has been suggested that the decreasing magnitude of the shunt in the ductus arteriosus may also play a role in reduction of the heart volume (49)

Studies in anoxic newborns (9, 70) and in infants with pulmonary atelectasis (71), suggest that under these conditions the heart either remains abnormally large or increases in size, presumably as a result of the high vascular resistance in the atelectatic or insufficiently aerated lung

Electrocardiographic Changes

Technical problems have thus far limited the clinical application of foetal electrocardiagraphy (78, 79). Recordings obtained at abortion with

dex and India ink) under a pressure of 80 mm. Hg into the capillaries through the pulmonary artery Against this view, it may be questioned whether the use of such a high pressure would make this mechanism applicable under normal conditions. It is possible that such a mechanism may assist in the expansion of the lungs at birth under normal conditions or in cases of asphyxia where the onset of air intake is delayed. The elastic recoil of the chest wall resulting from its compression in the birth canal generates a negative pressure which may contribute to the initial aeration of the lungs (62). As a consequence of the compression of the chest wall during birth, fluid is forced out of the lungs to be replaced by air. Serial film studies in our laboratory have shown that this is sufficient to fill the upper respiratory passages prior to the first breath of the infant. In addition, it has recently been possible to show, using rapid exposure x-rays, that in the newborn infant the initial inflation of the lungs is associated with a sequential distension and compression of the pharynx cavity similar to that which is seen in the glossopharyngeal breathing of respiratory-deficient poliomyelitis patients [Bosma *et al.* (63)] It is suggested that these movements may prevent egress of air from the lung and, thus, with an increase in intrathoracic pressure prior to the impending expiration, facilitate further distribution of air within the lungs.

For the present, we must accept as the major factor in lung expansion the active inflation under negative pressure of the lungs with air. As a result of this and its attendant opening of the pulmonary vascular bed, resistance in the pulmonary circuit drops, the pulmonary flow increases, and the pressure in the pulmonary artery falls. Ardran and his co-workers (77) were able to demonstrate an immediate drop in pulmonary arterial pressure on ventilating the lungs of the foetal lamb. Dawes *et al.*, also working with lambs, have shown that with the initial ventilation of the lungs the pulmonary flow immediately increases to four times the control value and the pulmonary resistance decreases to one-tenth its previous level. This is accompanied by a drop in the pulmonary arterial pressure (21, 64). They further showed that this fall in pulmonary resistance and pulmonary arterial pressure was caused by expansion of the lungs with a gas (air, oxygen, or nitrogen), the changes being the same regardless of which gas was employed. However, distension of the lungs with a liquid (saline) had the opposite effect of increasing the pulmonary vascular resistance and decreasing the flow They suggest that the differences arise from a direct mechanical effect on the lung vessels

The results of catheter studies of newborn infants show that the pressure in the pulmonary artery is higher than the "normal" value in older children and adults, and that it declines toward these "normal" levels by about 14 days of age (49, 53) Since we know that the lungs of the normal newborn infant expand almost completely within the first few minutes after birth (57), it is evident that there must be other factors contributing to the maintenance of the pulmonary arterial pressure Rowe (65) has pointed out

Some Problems of Adaptation to Extrauterine Life

In any consideration of the neonatal adaptation to extrauterine life, it is not possible to separate respiratory from circulatory function, as the two are intimately connected and mutually interdependent.

Anoxia is known to cause an increase in the pressure in the pulmonary artery in adults [Motely *et al.* (75)] Adams *et al.* (87) found no consistent changes in pressure in the pulmonary artery in response to the administration of a mixture of 10 per cent O_2 and 90 per cent nitrogen in normal infants less than 36 hr of age. The response was variable both in magnitude and direction. Similar findings resulted from the administration of 100 per cent oxygen.

James & Rowe (74) investigated this in older infants ranging from two days to several months of age. They found that in infants older than one month of age, induced hypoxia resulted in an increase in pressure in the pulmonary artery. The pressure changes in the aorta at this time did not appear to be significant. However, in infants less than one month of age, the rise in pulmonary arterial pressure was accompanied by a fall in aortic pressure, in some instances below the level of the pulmonary artery, and sufficient to produce a reversal of flow through a still patent ductus. In addition to the finding that less desaturation of the inspired air was needed to produce the change in the younger group, it was also noted that a similarly reduced O_2 mixture caused a much more precipitous drop in peripheral O_2 saturation in the younger group. The explanation for this difference lies in the fact that in the younger group with a still patent ductus arteriosus the reversal of flow in it, caused by the anoxic pressure changes, will allow venous blood to enter the aorta, magnifying the resultant peripheral desaturation

Finally, it is perhaps interesting to reconsider previous concepts of the etiology and pathogenesis of the cyanosis accompanying respiratory distress in the newborn Conditions interfering with lung expansion and aeration (atelectasis, infiltrations, alveolar capillary block, etc) will increase the resistance in the pulmonary circuit. As a result, there may occur a reversal of flow in the ductus arteriosus (i e, from right to left) When this happens, the venous return to the left atrium diminishes and the left atrial pressure may fall below that in the right atrium (already elevated as a result of the increase in pulmonary resistance) Under these circumstances, the foramen ovale may reopen and venous blood will enter the systemic arterial circulation (9). To a degree, this shunt is a compensatory haemodynamic adjustment on the part of the infant for it attempts to correct for the unequal volume of return to the two atria, even though this is accomplished at the expense of placing unsaturated blood into the peripheral circulation

the umbilical cord still attached to the placenta *in utero* suggest that the human foetus has a fully differentiated ECG at a relatively early age (80). Zitka & Papez (81), and Stern & Lind (82) obtained ECG's from infants before and after the first cry. No changes was noted in the format of the ECG with the first cry, although it was suggested by the latter group that asphyxiated children showed evidence of a greater strain on the right side of the heart (82).

The electrocardiogram of the newborn infant normally shows evidence of right ventricular preponderance (83, 90) which disappears gradually over the first year of life Because of this, the assessment of pathological degrees of ventricular hypertrophy during this period is difficult, especially in cases where the degree of change is not marked. The assumption that the lessening of the electrocardiographic right-sided preponderance is the result of the postnatal development of the left ventricle (38), has recently been challenged by evidence that there occurs as well an actual involution of the wall of the right ventricle amounting to as much as 20 per cent of its mass (72, 73).

Much attention has been paid to the curious behaviour of the T waves in the ECG of the newborn infant Thus, Ziegler (83) and Sodi-Pallares *et al.* (84) have described the changes in the right precordial lead V_1, where the T wave changes from a positive to a negative deflection within the first 24 to 48 hr. of life, to remain as such throughout infancy and childhood Recent studies of our own confirm this finding but would indicate that the above changes may occur as early as one-half hour after birth (85). Dupuis *et al.* (86) have been able to reverse these changes partially or completely by injecting epinephrine in newborn infants betwen 5 and 10 days of age As these changes normally occur at a time when the pulmonary arterial pressure is said to be declining, and as epinephrine is known to cause an elevation of the pulmonary arterial pressure in the newborn (87), it would seem possible that these T wave changes are in some way related to the normally falling pressure in the pulmonary artery.

It has hitherto been pointed out that the standard limb lead ECG of the neonate was characterized by low amplitude T waves (88, 89). Tracings taken in our laboratory immediately after birth (20 sec of life) indicate that the newborn standard ECG shows good amplitude T waves in the limb leads which tend to flatten within 30 min. after birth. Preliminary observations suggest that these changes, too, are reversible by the administration of epinephrine (85).

Asymptomatic rate, rhythm, and conduction disturbances evidenced on the ECG have been reported in a significant proportion of apparently normal newborn infants [Michaelsson (89)] These disturbances are confirmed by our own findings, and would appear to cause no clinical embarrassment to the infant and to disappear spontaneously without any treatment over a period of a few days

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KIDNEY DISEASE: ACUTE RENAL FAILURE[1]

By John P. Merrill, M D.[2]

Harvard Medical School, Peter Bent Brigham Hospital, Boston, Massachusetts, and Howard Hughes Medical Institute, Miami Beach, Florida

The subject of acute renal failure is an important one. It is important because this condition may be one of the most critical with which the physician must deal and yet it is also compatible with complete and lasting recovery. It is unfortunate, therefore, that the term "acute renal failure" has led to so much semantic confusion Acute renal failure, in the functional sense, has been confused with acute urinary suppression and acute renal insufficiency. From an anatomical standpoint it has been variously classified as shock kidney, crush syndrome, acute tubular necrosis and first, but least accurately, as "lower nephron nephrosis" (1). The word "failure" is defined by Webster's Collegiate Dictionary, fourth edition, as "omission to perform," and "insufficiency" as "wanting in strength, power, capacity; incompetent". These definitions certainly imply functional parameters The functional aspect seems important. For instance, one recent excellent review (2) states that acute glomerular nephritis cannot be included in the category of "acute renal insufficiency." However, in functional terms which is the ultimate concern of the patient, if not the semanticist, acute glomerular nephritis may certainly produce any and all degrees of "incompetence" of the kidney. Acute urinary suppression, on the other hand, would seem to imply the suppression of urinary function by influences from without the kidney. Whether or not this is semantically justified, it is convenient from a diagnostic standpoint to divide the general subject of acute renal failure into (*a*) prerenal (*b*) renal, and (*c*) postrenal. This division, in turn, implies that only in category (*b*) is there renal parenchymal damage and, by the same token, prerenal or postrenal defects may mask a kidney whose functioning parenchyma is intact This has important connotations for immediate diagnosis and treatment A further division that has seemed useful in the past (3) has been a division of acute renal insufficiency and acute renal failure. Acute renal insufficiency is defined as inability to perform standard tests of function, whereas acute renal failure implies inability to excrete at normal plasma levels the load of metabolite presented. As will be pointed out, acute insufficiency may appear to be failure when the metabolic load becomes so great that even the normal functioning kidney cannot eliminate it at normal plasma levels. This fact was pointed out by Addis (4) in citing the fact that the blood urea nitrogen may rise dramatically in normal young men whose

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blood or extracellular fluid is sequestered in the body, changes occur in the

mild stimuli of the sort mentioned, the urine may decrease in volume and become more concentrated, possibly as a result of liberation of antidiuretic hormone. Sodium excretion tends to drop, probably as a result of ill-defined influences upon pressor receptors elsewhere in the body which operate to cause differential shunting of blood through the kidneys without marked change in overall renal blood flow. With more potent stimuli, such as further decrease in "effective" intravascular volume or extracellular fluid, renal blood flow and filtration rate may drop In a teleologic sense, blood is now shunted from the kidney to the more vital organs such as the brain, and in this fashion arterial pressure and, more important, central nervous system blood flow may be maintained at the expense of renal blood flow (12). This may be accompanied by a marked decrease in urine volume and sodium excretion. Experimentally, this was first described in the experiments of Mueller *et al.* (13) who pointed out that decreased urine volume and sodium excretion might occur with constriction of the renal artery which was mild enough to produce little or no demonstrable drop in filtration rate This particular phenomenon is the basis for the so-called Howard test (14). When the filtration rate was reduced experimentally by renal arterial constriction of 30 per cent or less, Levinsky (15) showed that a marked decrease in urine volume occurred with increase in urine concentration and a decrease in sodium excretion, even in the absence of antidiuretic hormone action. Further decrease in filtration rate (15) then resulted in decreasing urine concentration even in the presence of maximal antidiuretic hormone. All of these events may be explained in terms of the "counter current multiplier" theory of urine concentration (16). In this view the substantial reduction in glomerular filtration results in almost complete sodium resorption in the proximal tubules. Therefore, dilute urine is obtained because inadequate amounts of sodium reached the concentrating site for the maintenance of an optimal counter current osmotic gradient. Decrease in the volume of tubular fluid results also in increased back diffusion of urea and a rise in BUN. All of these events may occur without ischemia sufficient to cause parenchymal damage since the urinary findings are completely reversible when constriction of the renal artery is released. Indeed, the data given by Phillips (17) suggest that renal plasma flow must drop to less than 5 per cent before renal parenchymal damage occurs (as evidenced by decreased extraction of PAH). Thus, many stimuli resulting from decreased "effective" blood volume may lead to a sequence of events characterized by oliguria, decreased sodium excretion, and urea retention without renal parenchymal damage. These manifold events characterize the "prerenal" phase of renal failure. In the period before renal ischemia has led to tubule

protein intake is doubled or trebled. A further division of renal failure into prerenal, renal and postrenal failure has the advantage for the clinician of implying that for prerenal and postrenal failure an etiologic factor exists which may be specifically treated Renal failure per se, as distinct from the others, implies that damage to the kidney parenchyma has occurred, damage which might heal by itself. In this situation, injudicious attempts to "force" the kidney to perform its urinary function may result in overhydration, pulmonary edema, and death. It was the recognition of this latter fact that, more than any single factor, has lead to the striking decrease in mortality in this syndrome. The general principle was first heralded by the publication of Strauss (5) in 1948.

It is the experience of those who have dealt most with acute renal failure that 30 per cent or more of the cases cannot be attributed to any single etiologic agent (3, 6). This is because any single agent or insult of the same potency will not consistently produce the same renal damage. The state of the organism at the time the insult occurs conditions the amount of damage. This situation may be likened to the physiologic effect of a given dose of adrenal steroid. In an untreated Addisonian patient it may cause primarily sodium retention, and in a normal individual, potassium excretion Thus, one individual may endure an hour and a half of hypotension with no renal functional impairment. In another, a transient episode of syncope associated with hemetemesis may yield marked renal failure. In all probability, the difference here is the difference in renal blood flow resulting from the drop in blood pressure. Unfortunately, the blood pressure cuff gives us only poor inferential evidence about the state of the blood supply to the kidney.

The causes of acute renal failure are multiple. These have been adequately covered by many previous reviewers (2, 3, 7 to 10) and will not be discussed in detail here. Rather, this review will limit itself to a condensed list of causes of acute renal parenchymal failure and to some discussion of the general principles involved in its production, recognition, and therapy. Because of the diagnostic and therapeutic importance of prerenal and postrenal factors which may be immediately remediable, these should perhaps be discussed first and in more specific detail.

Prerenal Failure

An understanding of the sequence of events which follows upon renal circulatory insufficiency is important, because recognition of this situation (herein described as prerenal failure for want of a better term) may lead to its correction before renal parenchymal damage has occurred. Renal circulatory insufficiency per se may be correctable but we can do nothing to reverse the process once tubular necrosis has occurred. The literature on extrarenal influences which modify renal function is massive. It has been recently reviewed by Homer Smith (11). Ordinarily, the kidney receives 25 to 30 per cent of the cardiac output. When the output falls or when

has been reported also in renal failure secondary to diabetic coma and transfusion reactions (24, 25).

Postrenal Failure

Postrenal factors operating to cause acute urinary suppression constitute a small but important percentage of the causes of acute renal failure. In acute renal failure caused by parenchymal damage or to pre-renal causes,

absolutely characteristic, story of obstructive uropathy in renal failure is total anuria followed by 24 hr. or more of large urine volume, with a sudden return to anuria. In renal failure from parenchymal causes, urine volumes do not behave in this fashion. Obstruction of one ureter with a non-functioning contralateral kidney may result in total anuria Renal calculi and, less commonly, carcinoma of prostate or cervix may present with such a history. One type of so-called "reflex" anuria has been ascribed by Sirota & Narins (26) to edema and obstruction of the uretero-vesical orifice. This kind of obstructive uropathy may occur after catheterization of the ureters. We have seen it also arising from edema higher in the ureters. Chronic pyelonephritis is frequently attended by changes in the ureter, and acute exacerbations may be accompanied by an ureteritis in an edematous, patulous ureter which may cause obstruction either uni- or bilaterally. This, in turn, may be aggravated by catheterization. Obstruction of the ureters attributable to the deposition of sulfonamide crystals is now rarely seen It should, however, be kept in mind. Obstruction of the ureters ascribed to the deposition of uric acid crystals either in the treatment of gout (27) or following the use of radiation or the alkylating agents in the treatment of leukemia or Hodgkin's disease, may also result in obstruction of the ureters with uric acid crystals when alkalinization of the urine and adequate urine volume have not been maintained (28, 29, 30). Ligation of the ureters in the course of pelvic surgery or retroperitoneal hematomas obstructing the ureter have also caused obstructive uropathy (3). Patients with chronic prostatism may develop acute urinary retention following the use of ganglionic blocking agents or even antihistamines.

Obstructive uropathy, though rare, should always be considered since it is one of the immediately reversible causes of urinary suppression. The history should suggest a possibility of one of the causes mentioned above. Injudicious investigations of the upper urinary tract by bilateral catheterization is to be condemned. When, however, a reasonable possibility of obstructive uropathy exists it must be employed to rule this out. A good general rule seems to be the following: A plain film of the abdomen should be obtained If two normal sized or enlarged kidneys are visualized and historical evidence for obstructive uropathy is obtained, the ureter (on one

necrosis this sequence may be reversible by correction of the underlying dynamic defect.

The diagnosis of prerenal failure, therefore, may be suggested by the appearance of oliguria, a marked decrease in urinary sodium concentration and an increase in total urine concentration as measured by its osmolality. Concomitantly some degree of nitrogen retention may occur. From the clinical standpoint a history of antecedent events leading to decreased "effective" intravascular volume supports the presumptive diagnosis

Unfortunately, there are pitfalls in applying these phenomena to the clinical diagnosis of acute renal failure. Diminished urine volume alone is not an adequate criterion. Oliguria can occur with perfectly normal renal function. Urine volumes of 400 ml. per day with a urine specific gravity of 1.030 and osmolality of 1200 milliosmols per liter may occur in normal individuals with normal renal dynamics, purely as a result of water restriction. Drastic restriction of dietary protein and salt may reduce the amount of solute (urea and sodium) "claiming excretion" (18) so that even further diminution of urine volume may occur. If the urine volume continues at 400 to 500 ml. per day with minimal solute output, urine specific gravity may decrease to 1.014 or 1.016. Thus, oliguria may occur as a result of prerenal influences with either concentrated or dilute urine.

The blood urea nitrogen level may also be misleading since increased protein metabolism may result in increased urea loads which require excretion at higher plasma levels even with normal renal function. Increased protein metabolism is a common concomitant of burns, surgery, and infection, all of which are common precursors of renal or prerenal failure. Since, however, the level of renal function is reflected not by the plasma level alone but also by the clearance of urea, the simultaneous measurement of plasma level and the rate of excretion [urine concentration × cc. of urine per minute (UV)] may give a more precise estimate Perlmutter (19) has suggested that perhaps the ratio of urinary urea to plasma concentration may be of help in "differentiating the azotemia or oliguria of renal disease (<10) from that of dehydration or inadequate blood flow [(19, p. 714)]."

Several reports have appeared suggesting that the ability to produce urine with low sodium concentrations is inconsistent with the presence of continuing renal parenchymal damage and, conversely, that in urines of low volume, high sodium concentrations presuppose renal damage (20, 21, 22). These clinical observations are consistent with the data from the experimental work mentioned above This appears, then, to be a good general rule, and certainly a better index than urine volume or blood urea nitrogen concentrations The daily pattern of urinary sodium to potassium ratio may be even more accurate. The Na/K ratio is highest during the first few days of oliguria and falls steadily thereafter until the onset of diuresis (22). Exceptions, however, have been noted (19). One series of burn patients with renal failure persistently showed low concentrations (20, 23). This

cumstances whole groups of nephrons are totally destroyed or infarcted and the result is "cortical necrosis." Contrary to the older literature, kidney function may be resumed in cortical necrosis and the very patchy nature of the lesion accounts for the fact that it is compatible with survival (32). When the vascular lesion is sufficiently severe that all the glomeruli are involved, the renal cortices are involved in a diffuse or symmetrical type of lesion in which the entire cortex is infarcted. This is "symmetrical cortical necrosis" and, of course, is not compatible with survival. The ultimate step in renal ischemia is, of course, obstruction of the renal artery, in which case the entire kidney is infarcted

The sequence of events by which ischemia leads to acute renal failure is complex and poorly understood The fundamental importance of ischemia in the production of acute renal failure seems inescapable from a consideration of the kidney's role in diverting blood to other organs under various conditions of stress Unfortunately, in the human being at least, decreased renal blood flow is difficult to document by the conventional clearance methods *since little or no urine may be obtained. Even when small amounts of* urine are elaborated, measurements may be invalid because the extraction of the test substance [iodopyracet compound or paraminohippurate (PAH)] by the damaged kidney may be subnormal Some investigators have corrected for this abnormality by catheterizing the renal vein in cases of human anuria and directly measuring the extraction of PAH by the Fick principle (21, 33). In the cases in which it was measured by this technique, the renal blood flow was uniformly found to be decreased. In recent years more elegant techniques have been utilized employing radioactive krypton (34). These studies have documented the existence of decreased renal blood flow in one human case of acute renal failure. Conn (35), measuring the renal extraction of nitrous oxide in dogs made experimentally oliguric, also found decreased renal plasma flow. The older work of Trueta *et al* (36) suggested that oliguria occurred by reason of a diversion of blood flow from the renal cortex to the medulla through the juxtamedullary glomeruli This work was largely done in rabbits and has been vigorously disputed, particularly in this country, as a causative factor in acute renal failure in man. Clark and his colleagues (37) found normal arteriovenous oxygen differences across the kidney in a case of human anuria and felt that the Trueta mechanism could not be operative under these conditions It is entirely conceivable, however, that the initial acute insult might well be accompanied by diversion of cortical blood through the juxtamedullary glomeruli just as described by Trueta. Cortical ischemia of a patchy nature would then result and redistribution of blood to the unaffected cortical tissue might account for the results reported by Clark The data submitted by Powers (38) showing fall in urinary oxygen tension in experimental postraumatic renal failure, are of interest. His observations are consistent with either the opening of shunts bypassing parts of the nephron, or with the redistribution of red

side only) should be carefully catheterized to the renal pelvis. No retrograde pyelogram need be made. If, from the normal sized kidney catheterized, no urine is obtained, obstructive uropathy can be presumed not to be the basis of acute renal failure. It should be remembered that when the ureter is edematous the catheter may act as a splint and while in place may allow adequate urine flow from the renal pelvis. When removed, obstruction may reoccur. The ureteral catheter must then be left in place until inflammation and edema have subsided.

Renal Failure

The term "renal failure" is used in this review to denote failure of the kidney to excrete at normal plasma levels the metabolic load presented. When used without the prefix "pre" or "post" it will henceforward connote damage to the renal parenchyma. The pathogenesis of renal parenchymal failure can be divided into two major groups: (*a*) ischemia, and (*b*) nephrotoxins. The anatomical lesion resulting from these two causes has been described in the classic studies of Oliver (31). He studied these lesions in preparations in which the entire nephron was separated by microdissection. His studies suggest that the lesion produced by a nephrotoxin is that of uniform necrosis of the tubular epithelium down to, but not including, the basement membrane. When ischemia alone is the cause of the renal failure there may be complete disruption of the renal tubular structure with fragmentation of the basement membrane and leakage of the tubular contents into the interstitium. This latter lesion has been called by Oliver tubulorrhexis. Oliver's studies showed that the designation "lower nephron nephrosis" applied in the earlier description by Lucké (1) was not appropriate since the ischemic lesion could occur in any part of the entire nephron. The descriptions by Oliver and other pathologists stress the fact that many nephrons in injured kidneys are left uninvolved. Healing of the lesion, according to Oliver, involves the resorption of edema and repair of tubular epithelium. When the basement membrane is intact the epithelial cells regrow along this structure, but where it has perforated complete disruption of the tubular continuity might occur with secondary ingrowth of connective tissue elements. The nephrotoxic lesion and the ischemic lesion were not completely separable since, in many of the kidneys damaged by nephrotoxins, the lesions typical of ischemia might also occur.

The glomeruli were characteristically uninvolved. Since the blood supply to the renal tubules comes by way of the glomerulus it seems reasonable that decrease in renal blood flow should first involve the postglomerular tissue, (i.e., the tubules) and that, to some extent, the degree of tubular damage reflects the degree of ischemia. Characteristically, however, this damage is scattered with areas of tubular damage interspersed with areas of apparently uninvolved tissue. The damage may still be patchy even when the ischemia is so severe that the glomerulus itself is involved. Under these cir-

sure in a case of human acute renal failure. However, these techniques are open to question and there is a wide variation in the results of other investigators citing normal renal interstitial pressure. It is unlikely that the renal interstitial edema would effect all nephrons equally since the nephron population is believed to be a heterogeneous rather than a homogeneous one (49). A given increase in interstitial pressure would then occlude some nephrons before others and might well result in the "patchy" nature of the lesion. Occlusion of tubules by edema with or without plugging by casts would result in increased back pressure and decrease in overall filtration rate. Similar[redacted]essure occlusion of the peritubular capillaries would result in decrea[redacted]nal blood flow. Further support is derived from the data provide[redacted]nshaw which suggest that the effective driving force for blood flo[redacted]s the difference between renal artery and tissue pressure. Therefore, an increase in tissue pressure such as might occur with edema would cause a decreased blood flow, other things being equal (50) One might expect that, if renal edema were causative, removal of the restricting capsule might result in improvement This has not been the case (3, 51). Previous reports of favorable results of this procedure have probably not been cause and effect because "in a condition like acute anuria which is so dramatic for both patient and doctor human patience with regard to desisting from intervention seems to last about as long as the oliguric state of the disease" (10) There is, however, other inferential evidence from clinical observations The increasing volume of urine and, occasionally, the sudden onset of diuresis with urine volumes increasing by 2 liters from negligible output is much too rapid to be explained by the healing of ischemic tubular necrosis. It is not, however, too rapid to be explained by increase in the ratio of intratubular to interstitial pressure allowing normal tubules previously occluded by increased interstitial pressure to become patent and allow urine flow The prevention of experimental acute renal failure by the use of osmotic diuresis which again increases the gradient, further favors this idea. Finally, a fortuitous observation of the author seems pertinent. In the transplantation of a normal kidney from a healthy identical twin to his sick sibling, the renal artery and vein are clamped simultaneously and the vessels severed A period of 45 min. to 1.5 hr may ensue before blood flow is resumed These kidneys begin to elaborate urine almost immediately when the renal arterial clamp is released in the recipient. If ischemia per se were the sole cause of acute renal failure this should not be so. In one instance of transplantation, however, the renal artery was clamped, released for a moment and then reclamped. This kidney when transplanted was swollen, tense, and showed patchy cortical ischemia. Urine flow under these circumstances did not begin for 36 hr. These observations in the human suggest that possibly the seqence of clamping, release, and clamping allowed differential renal vasoconstriction which, with resumption of blood supply before severence of the renal artery, allowed edema for-

cell rich blood as postulated by the "plasma skimming" theory advanced by Pappenheimer (39). It is difficult to refute this possibility when one sees at the autopsy of a patient dying from acute renal failure kidneys with patchy cortical ischemic lesions and a congested medulla. This sequence of events would be entirely consistent with the observations of many observers that glomerular filtration continues (33, 40). As early as 1929, Richards reported by direct observation of the frog kidney poisoned with mercury that active filtration occurred in spite of virtual absence of urine formation (41). Sims (42), using a fluorescent dye to stain glomeruli in rats whose kidneys had been damaged by mercuric chloride, showed presumed continued filtration by staining of the glomeruli under conditions of severe suppression of urine volume.

If, then, glomerular filtration continues, what becomes of the filtrate if no urine is formed? One school of thought holds that the explanation for this strange phenomenon is non-selective back diffusion through damaged tubules whose structural integrity may be represented only by basement membrane. If this were true it would be important to have data showing molecules of different molecular size and configuration diffusing at different rates Some data exist suggesting that the ratio of creatinine to urea clearance may by altered in the various stages of acute renal failure (43) and similar data have been published for PAH and ferrocyanide (44). Others, however, disagree (21, 40) A gross leak through a rip in the basement membrane would cause back diffusion without any differential and this is suggested by the work of Oliver quoted above. This might, perhaps, be abetted by plugging of the tubule distal to the "leak" by casts which, though not in themselves the cause of the renal failure, might favor further leaking into the interstitium. One might well ask, "If filtrate is formed and diffuses back into the interstitium, where does it go?" No firm answer to this question is forthcoming, but the possibility that it might be transported back into the blood stream by way of the renal lymphatics has been raised (45). This is suggested as "one more out" by no less a scholar of things renal than Homer Smith (46). The matter is far from settled, but in the opinion of the present reviewer, the role of edema in the causation of acute renal failure is a major one. Edema is a secondary, not a primary, factor and results as it does elsewhere in the body from ischemia and necrosis. The early biopsy material of Iversen & Brun (47) showed edema of the interstitial tissue However, much of the other biopsy material in cases of acute renal failure, including those of the author, have shown remarkably little pathologic change early in acute renal failure This, however, is not unexpected in view of the patchy nature of the lesion Direct measurements of renal interstitial pressure in dogs with experimental acute renal failure have shown no striking increase (48). One of the techniques of cardiac catheterization applied to the measurement of "wedge renal venous pressure" produced data interpreted as showing no increase in renal interstitial pres-

of normal nephrons may be allowed to resume function. Occasionally one sees a rapid increase in the volume of urine which after 4–5 days of marked oliguria may suddenly reach 2,000 to 2,500 ml per 24 hours In the absence of obstructive uropathy this is better explained by resorption of interstitial edema and resultant decompression of undamaged nephrons than it is by the process of epithelial repair in damaged tubules (3)

It was suggested that a large volume of dilute urine under these circumstances might represent a profuse osmotic diuresis resulting from the relatively marked increase in filtered solute presented to the few normally functioning tubules Recently this idea has received further support from the observations of Meroney & Rubini (22) who have shown that in the diuretic phase of acute renal failure the renal tubules may selectively regulate the concentrations of sodium, potassium and nitrogen in such way that although the urine remains isotonic to plasma the concentrations of these various constituents change in a reciprocal fashion

The foregoing, to be of value, should have more than theoretical interest. It should, and does, give clues to the prophylaxis and treatment of acute [illegible]
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Ganglionic blockade, which should, at least in part, prevent renal vasoconstriction, has been shown to prevent the development of renal damage in mallet trauma (38), experimental placental abruption (61), and aortic occlusion (62). This has been demonstrated to prevent renal damage and decrease in renal blood flow in humans subjected to procedures lowering the the systolic blood pressure (63). Perhaps more striking are the better controlled experiments in dogs in which lidocaine hydrochloride (Xylocaine) injected into the renal artery before clamping of the aorta minimized the damage on the side injected and not to the uninjected kidney (64). Evidence published by Powers and his collaborators (62) suggested that the intravenous infusion of trimethaphan camphorsulfonate (Arfonad Camphorsulfonate, a ganglionic blocker) might be useful in the prevention of renal failure following aortic surgery, a situation in which acute renal failure is a notorious and unfortunate sequence. If the flow of tubular urine is maintained the gradient of intratubular, extratubular pressure should also be maintained General anesthetics and preinduction narcotics may result in antidiuresis and decreased urine flow This, in turn, possibly accounts for the frequency of acute renal failure following surgical procedures or burns (117) in which adequate hydration is not maintained. Furthermore, the use of mannitol, ~~dextran, or~~ hypertonic urea solutions (118) during or immediately following the surgical procedure or burn, appears to decrease the incidence of acute renal failure, and this may well be accomplished by the diuretic effect of both of these agents It should be stressed at this point

mation which did not occur when the renal artery was clamped and severed before this could occur.

The role of the nephrotoxins deserves little further mention. Poisons filtered or secreted into the tubular urine and reabsorbed may damage renal tubular epithelial cells in their passage. The necrosis of tubular epithelium particularly in the proximal tubule is specific for nephrotoxins but the ischemic damage that follows is strikingly similar to the renal lesion of shock. The role of blood and muscle pigments as nephrotoxins per se is now generally discredited. The elegant microdissections performed by Oliver have shown no relation between the localization of pigme[illegible]sts and tubular lesions (31). The infusion of pure hemoglobin pigm[illegible]rmal animals or humans does not produce acute renal failure even i[illegible]amounts (52, 53). While the intravenous infusion in man of large amo[illegible]s of distilled water or hemolyzed autogenous blood (54) may produce renal ischemia and oliguria, this is probably a result of an increase in peripheral vasoconstriction and a fall in cardiac output rather than a toxic action of the pigment itself (53). In the case of distilled water infusion the oliguria is accompanied by decrease in renal blood flow and filtration rate which can be reversed by the vasodilator effect of parathormone. Vasodilator agents employed at the time of the infusion of red blood cells may also prevent such renal ischemia in dogs (55) One of the common causes of acute renal failure in man is the administration of incompatible blood. This is associated with hemoglobinemia and hemoglobinuria, and at one time it was thought this pigment was responsible for the renal lesion However, Castle (56) has demonstrated the obvious fact that *in vivo* as *in vitro* incompatible bloods first agglutinate when mixed. It is probable that this agglutination causes capillary stasis, ischemia, anoxia and that the red cells are hemolyzed in this environment after first causing the vascular damage.

It has generally been impossible to produce consistently in the experimental dog a renal lesion by the infusion of hemoglobin or methemoglobin alone. One must use in addition severe dehydration (57) or clamping of the renal artery (58) and pitressin (59). The one exception to this appears to be the recent observations of Teschan (60) who has produced renal failure in rats without prior ischemia by the administration of methemoglobin and sodium ferrocyanide. It seems probable that the role of heme pigments in the production of acute renal failure is a minor one and secondary to the ischemia produced Whether or not the heme pigment casts make some contribution to the oliguria by occlusive plugging of renal tubules is a matter of conjecture.

These ideas were summarized several years ago as follows:

> The lesion in acute renal failure is scattered among groups of nephrons as well as in varying sections of the individual nephron Many segments of the kidney may be spared The resorption of interstitial edema may coincide with the onset of the diuretic phase after anuria and under these circumstances the scattered groups

Clinical Course and Treatment

The description of the clinical course of acute renal failure and the principles involved in the therapeutic approach have been discussed in some detail by previous reviewers (2, 3, 7, 8, 10, 69, 70). The present writer will outline only briefly the factors discussed by these authors but will dwell more particularly upon recent additions or corrections.

counts for the more rapid rise in the non-protein nitrogen concentration

been confirmed by more recent work (73, 75). The expansion of extracellular space found by recent workers (73) may result from this as well as from injudicious fluid therapy. Hyponatremia has been a frequent concomitant of acute renal failure but as Swan & Merrill pointed out (7) this is frequently the result not of sodium loss but of dilution caused by excess water administration. This observation, too, has been borne out (73). For some years it has been the fashion to speak of "shifts" of sodium and potassium between extracellular and intracellular compartments. Acidosis and the overall metabolic defects of uremia [probably by impairing the generation of high-energy phosphate bonds for the metabolic pump (74)] contribute to this, and measurements of total body potassium by the isotope technique have suggested that potassium intoxication with high extracellular levels may exist in the face of total body deficit. In the experimental dog made anuric by ureteral ligation, muscle sodium and chloride concentrations have been found to be elevated (75) On the other hand, it seems probable that hyponatremia in acute renal failure is usually in large part attributable to excess water administration. Although the specific instance of acute renal failure was not studied by Edelman and his co-workers (76), in many other abnormal clinical situations serum sodium concentrations were found not to be a function of intercompartmental shift but of the relation of total exchangeable sodium, exchangeable potassium, and total body water. The significance of these findings for the clinician appears to be that water restriction should be greater than that originally proposed by Strauss (5) For the average 70-kg. adult, 300 to 400 mls. of water per day is the baseline requirement. To this, of course, should be added increments for gastrointestinal losses, increased respiratory rate, high fever, and profuse sweating Daily measurements of the body weight continue to be the best gauge of adequate replacement therapy Ideally, in the face of our inability to supply adequate calories, a weight loss of 0 2 to 0 3 kg. per day should be effected. In some instances this may be slightly more than necessary But

that these measures must be employed prophylactically since, once tubular necrosis has occurred, no amount of osmotically active substance or vasodilating agents will increase urine flow.

It seems logical that anything increasing vascular reactivity or predisposing to renal vasoconstriction would facilitate acute renal failure for reasons mentioned above. Acute renal failure is a common complication of abnormal pregnancies (65). While it constitutes only a small percentage of the problems seen in general obstetrical practice (66), it nevertheless constitutes more than 20 per cent of patients referred to centers for the treatment of acute renal failure (6). Uterine distention which may occur during prolonged or difficult labor has been shown to exert vasoconstrictor influence on the kidneys of animals (67), and placental abnormalities with premature separation are thought frequently to give rise to a Shwartzman phenomenon involving the small vessels (68). All of these factors would favor renal vasoconstriction and ischemia, particularly in a setting of blood loss in which diversion of blood from the kidney and decreased renal blood flow may have already occurred. The pathophysiology of acute renal failure and the physiologic basis for its prophylaxis have been discussed in some detail, since it is in this parameter that thinking has changed most radically in recent years The clinical course, complications and therapeutic principles have been considered at length in a number of previous reviews, and for this reason only supplementary information will be stressed in what follows

The difficulties in making a definitive etiologic diagnosis of acute renal failure have already been stressed. A few hints from a large clinical experience, however, may be worthwhile. Oliguria following the inhalation or ingestion of carbon tetrachloride may not occur suddenly but may develop over a period of days. Because it goes unnoticed by the patient it may frequently be confused with acute glomerulonephritis. Frequently such patients may have abdominal pain, pulmonary edema and hematuria with nausea and vomiting as initial symptoms In the author's experience this is the only nephrotoxin which may give rise to red cell casts Acute renal failure following the use of distilled water in the irrigating fluid of transurethral resections of the prostate, formerly a frequent source of patients, has become almost non-existent since the institution of the use of osmotically active nonelectrolytes in the irrigating fluid. Bichloride of mercury poisoning is frequently accompanied by severe bloody diarrhea and produces a renal lesion which, unlike other forms of acute renal failure, may result in permanent renal insufficiency. Bacteremia with *Clostridium welchii* in septic abortions may characteristically produce cyanosis and hemoglobinemia with severe myalgia, high fever, and methemoglobinemia as most typical concomitants accompanying acute renal failure. Even more characteristic is the denial by the patient of any attempts at instrumentation of the uterus.

hemodialysis with the artificial kidney (89). The various cation exchange resins have been effective in removing potassium from the intestinal contents. Difficulty in getting these to the intestinal tract when given by mouth or in effecting their return when given by enema have until recently limited their uses Recently, a smaller mesh sodium cycle resin with less tendency to the production of impaction has been made available (Kayexalate · Winthrop Laboratories, Inc.). Given by mouth to nephrectomized dogs, it significa

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gastrointestinal tract will be metabolized (91).

The Provision of Calories

The "forced feeding" of fat and sugar mixtures to suppress protein metabolism (92) has now been largely abandoned in the treatment of acute failure because of the difficulty of inflicting such a regimen on acutely ill, usually nauseated, patients The use of intravenous fat emulsions to provide calories has, in the past, been fraught with the hazard of pyrogen reaction. But recent preparations may obviate this Since, however, renal failure is a self-limited disease and since body fat stores are usually adequate for the period of anuria, it does not seem necessary to emphasize the administration of fat in spite of its high caloric value Where possible, calories should be given by mouth in order to minimize the amount of fluid necessary and to provide for the mouth, salivary glands and oropharynx, the necessary stimulation of drinking and swallowing. This appears to be important in minimizing the oral lesions which so frequently accompany acute renal failure Small amounts of Karo syrup and ginger ale mixed in equal proportions and well iced are of benefit when tolerated Frequently, however, it is impossible for the patient to take anything by mouth either because of surgical complications involving the gastrointestinal tract or because of severe nausea. Under

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of a plastic catheter in veins of large enough caliber to prevent thrombosis or phlebitis Catheters placed through the femoral or saphenous vein into the inferior cava are frequently associated with thrombosis and infection, and this in a most hazardous area (93, 94) When threaded through an antecubital vein into the cephalic or brachial vein and changed every five to six days embolic complications are rare The importance of providing a constant source of calories over a 24-hr period in this fashion seems to more than offset the potential risk. The provision of vitamin supplements is probably of benefit and certainly of no harm. Attempts to minimize protein metabolism by the use of anabolic agents is generally practiced (3)

it is a sound principle of therapy that it is easier to administer fluid to the dehydrated anuric patient that it is to remove it from the overhydrated individual with actual or incipient pulmonary edema.

With increased awareness of the principles of therapy the hazard of acute potassium intoxication is less frequently seen in the anuric patient than previously Potassium liberated in the process of increased tissue catabolism as well as potassium derived from devitalized tissues and extravasated blood, contributes to the increased incidence of potassium intoxication in military trauma (77). The increased incidence of infection in traumatized patients further contributes to this hazard (78). The necessity for drainage of accumulations of blood and pus, and the debridement of devitalized tissue is an important therapeutic point, not only from the purely surgical standpoint, but from that of the therapy of the "uremic syndrome" (79). It has been suggested that relatively more rapid rises in serum phosphate (80) and serum creatinine (81) than in blood urea nitrogen concentrations may suggest the necessity for the removal of devitalized muscle tissue This observation, however, has not been generally recognized as valid (6).

Since the cause of death in potassium intoxication is cardiac arrhythmia (82) the electrocardiogram is imperative in following patients with acute renal failure. The characteristic changes in the electrocardiographic patterns and their sequence have been described elsewhere (82, 83, 84). There is no direct correlation between the changes in electrocardiogram and the level of the serum potassium The complex which results in the disturbance *of cardiac conduction appears to depend upon the ratio of intracellular to* extracellular potassium which, in turn, may be affected by the pH of body fluid and the intracellular/extracellular sodium ratio. The treatment of advanced potassium intoxication is the infusion of hypertonic sodium bicarbonate. The antagonism of the potassium ion by the sodium ion and the beneficial effect of increasing pH exerts its action within a matter of minutes but is of relatively short duration and should be reserved for pa-
[illegible] chloride is

glucose with insulin in a ratio of 1 unit of insulin per 3 gms. of glucose *is more slowly effective but lasts somewhat longer* The removal of potassium by short-term intermittent peritoneal irrigation was described by Legrain & Merrill as an effective form of therapy for this complication in 1953 (85) This technique has been employed with success in a slightly modified form by subsequent workers (81, 87) The removal of potassium *from the gastric* contents by the simple inlying tube or by the more complicated process of "gastrodialysis" (88) may also be effective. The most effective treatment, however, for potassium intoxication, because it not only removes potassium but corrects the acidosis and metabolic defect which predisposes to it, is

modified by therapy. Hyper-reflexia, twitching and convulsions were formerly attributed to "hypocalcemia" which frequently occurs. In actuality, however, hypocalcemia per se is rarely the cause of these neurologic manifestations. Tetany may occur if alkaline solutions are administered rapidly by vein, but the central nervous manifestations in general are those of diffuse intoxication similar to that seen in hepatic coma. Indeed, the "liver flap" of the extended hands is not uncommon in uremic coma. Electroencephalographic studies show diffuse and gross abnormalities and correlate poorly with the presence or absence of a positive Chvostek's sign. Focal transient neurologic lesions may be present which can not be correlated with vascular or brain damage. Rarely do these abnormalities respond to the infusion of calcium salts They appear to be correlated generally with

INFECTION

Improvement in the management of the chemical manifestations of the uremic syndrome in acute uremic failure has exposed another and more ominous complication, that of infection The frequency with which acute renal failure now follows trauma of surgical procedures obviously predisposes such patients to infection. The prevalence of coma and vomiting makes them susceptible to aspiration of stomach contents and results in aspiration pneumonia (3, 6, 8). The frequency with which prophylactic antibiotics have been used in such instances have resulted in a disheartening incidence of "super-infection" with antibiotic-resistant staphylococci (97, 98). Infection, when present, should be treated promptly with appropriate antibiotics for which the sensitivity of the organism has been proved by *in vitro* studies. Care must be utilized in gauging the doses of antibiotics in the anuric or severely oliguric patient, particularly in the use of streptomycin and Kanamycin, for which a therapeutic blood level may be obtained for a period of two weeks with a single intramuscular dose of 1.5 gm (99, 100). The indiscriminate use of the inlying bladder catheter predisposes such individuals to pyelonephritis (101). Until the 24-hr. urine volume in the diuretic stage is greater than 1500 mls., it is rarely of clinical benefit Careful percussion of the bladder should reveal retention of this amount long before 1500 mls, has accumulated and, thus, for diagnostic purposes alone, the inlying catheter is unnecessary. The use of careful asepsis including mask and gown precautions may decrease the pathogenic flora to which the patient is exposed. Even though such precautions may not eliminate bacteria from the air or floor, they serve the useful purpose of making attending personnel aware of the constant problem of sepsis and contamination.

The administration of large amounts of intramusclular human gamma

Testosterone propionate in doses of 50 mg. per day for a week to ten days or the use of the more slowly absorbed depot testosterone (100 mg. once a week) are of unquestioned benefit in promoting positive nitrogen balance in healthy individuals. It is difficult to document the therapeutic value in acutely ill patients in whom no urine can be obtained to check nitrogen balance but it is the general impression that these agents may be of value In recent publications, English workers have stressed their belief in the efficacy of norethandrolone (Nilevar) in patients whose anuria follows accidents of pregnancy (95, 96).

Gastrointestinal Complications

Nausea and vomiting are almost constant concomitants. Marked diarrhea may be present. More frequently, marked ileus may be seen which, in combination with the nausea and vomiting and the leukocytosis, may closely simulate an acute surgical abdomen. The etiology of these disturbances in gastrointestinal function is not apparent. In all likelihood, it is related to the chemical abnormalities of "uremia."

Cardiac Complications

A frequent and disturbing complication of acute renal failure is cardiac arrhythmia. Such arrhythmias may result from sudden changes in serum electrolytes as a result of therapy. Therapeutic procedures which raise the serum sodium concentration and decrease the serum potassium are the commonest offenders. The various supraventricular tachycardias, particularly paroxysmal auricular tachycardia and auricular fibrillation are most commonly seen. Ventricular premature contractions occur even in undigitalized patients, and the ventricular arrhythmias may account for sudden unexplained death in patients with acute renal failure. A striking and unexplained phenomenon is the occurrence of a syndrome mimicking in every respect pulmonary embolus and acute cor pulmonale. Marked cyanosis, elevated venous pressure, dyspnea, and expiratory wheezes may be present. An electrocardiogram may show all the criteria for acute right heart strain, and yet, at autopsy, no pulmonary emboli are found Again, this bizarre symptom complex remains unexplained but appears to be related to the chemical abnormalities of "uremia," since dialysis with the artificial kidney or the onset of the diuretic phase may cause marked improvement.

Central Nervous System Manifestations

The central nervous system manifestations of acute renal failure are many and varied. Drowsiness and decreased responsiveness may progress to frank coma. On the other hand, the patient may be wildly agitated and disoriented The reflexes may be hyperactive or entirely flaccid depending upon the chemical abnormalities and the way in which they have been

phase. Convulsions and central nervous system disturbances are not infrequently associated with this stage but do not necessarily have a bad prognostic significance. With the central nervous system disturbances, hypernatremia may occur. This has been ascribed to a specific central nervous system lesion and its effect on the renal tubule, but in the author's experience it is more frequently a result of injudicious administration of hypertonic sodium solutions.

Extrarenal Removal of Metabolites

Since the fundamental problem in acute renal failure is the retention of normally excreted metabolites and electrolytes, the removal of these substances by extrarenal routes is a logical therapeutic approach. This has been accomplished by a number of methods.

Exsanguino transfusion.—This has been extensively studied by French workers (108). The removal of whole blood by phlebotomy from the uremic patient and its replacement by freshly drawn blood from normal compatible donors is the method employed. This procedure has a particular advantage in infants and small children where the volumes to be utilized are small. In the adult it is a somewhat more difficult technique. It has been advocated immediately after the occurrence of incompatible transfusion reaction since the products of hemolysis will be removed by that technique and not by dialysis across the peritoneal membrane or artificial membrane. It has been suggested that toxic molecules of large molecular weight which do not pass these membranes constitute a special advantage of this technique (109). Such molecules, however, have yet to be accurately identified Exsanguino transfusion is rarely used in the treatment of acute renal failure in adults today.

Cross transfusion.—The exchange of blood between an anuric patient and a normal donor by a direct connection between the two partners has received some attention (110) The problem of carefully adjusting the volumes exchanged and the possible hazards to the normal donor has eliminated this method as a practical therapeutic technique.

Intestinal perfusion.—Lavage of the intestine by means of a tube placed in the small intestine by the nasopharynx has, in the past, been extensively utilized particularly by French authors (111). This is less effective and more laborious than other techniques to be described and is little used today

Peritoneal irrigation.—The peritoneum is a semipermeable membrane of large area across which crystalloids and larger molecules will diffuse from the extracellular fluid into a solution placed in the peritoneal cavity. The removal of electrolytes and metabolites by this route has received considerable attention and constitutes an effective and relatively simple form of treatment when no surgical contraindications to irrigation of the peritoneal cavity exist. Continuous long-term peritoneal irrigation with the use of inlying sump drains (112) has now been abandoned because of the con-

globulin (5 to 10 cc. daily) may be an extremely effective adjunct in the antibiotic therapy of the infected uremic patient.

Anemia

Bone marrow biopsies from patients with acute renal failure show a striking diminution of erythroblasts but hypertrophy of myeloid elements consistent with the anemia and leucocytosis found in the peripheral blood (120). Survival of red cells infused into patients with chronic renal failure is decreased (121), although red cells from uremic donors survive normally in normal recipients. The anemia is a normochromic, normocytic one and may persist for weeks after the return of BUN to normal values. Reticulocytosis, often reaching 20 to 30 per cent, precedes the return of hemoglobin values to normal.

Each patient appears to stabilize at a hemoglobin value which represents a balance between hemolysis and red cell production. Transfusion of fresh packed red cells is the therapy of choice. Since the hemoglobin levels attained by transfusion may return rapidly to base line levels, for practical purposes it is usually necessary to maintain the hematocrit between 25 to 30.

Bleeding Defects

In spite of careful and exhaustive studies, a single cause for the bleeding defect in uremia, both acute and chronic, has not been found. Multiple and different defects in hemostasis have been described and to no single one can the frequent bleeding into skin and mucus membranes be ascribed (102 to 106).

The Diuretic Phase

The urine volume in recovery from acute renal failure usually increases in a stepwise fashion Rarely, it may increase suddenly from a volume of less than 300 ml a day to greater than 1500 ml Marked wasting of sodium has been described in this period (107) and may occur for one to two days. However, as renal function improves, sodium concentration may increase in proportion to the status of body sodium stores Frequently, marked sodium losses in the diuretic period reflect the loss of previously acquired edema fluid, and to replace these losses simply on the basis of urinary determinations is to perpetuate the edema A rare patient who enters the diuretic phase semicomatose and unable to respond to the stimuli of thirst and dehydration, may require intravenous therapy to prevent severe sodium and water losses Large losses of potassium may occur in the diuretic phase and the correction of acidosis at this stage may unmask a total body deficit which was hidden by elevated levels of serum potassium occasioned by the systemic acidosis The urine is hypotonic or isotonic to the plasma in the early part of diuresis. Since water is lost in excess of solute and since urea clearance is not adequate to keep up with protein catabolism, the BUN characteristically rises for the first few days and even weeks of the diuretic

from failure of elimination of metabolites the "artificial kidney" is a logical therapeutic method. Under circumstances in which it can be wisely and efficiently used, it may be employed to prevent the uremic syndrome in acute renal failure rather than to treat it once it has occurred. The use of "prophylactic" daily dialyses under these circumstances has recently been reported by Teschan (119). Although its use was viewed with some misgivings until recently, it is the opinion of those with most experience in the treatment of renal failure that the artificial kidney is of established value in the treatment of this syndrome, and that in proper hands it should be used more frequently in the future (6).

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stant occurrence of infection. Several techniques employing the intermittent instillation of the appropriate solution into the peritoneal cavity by means of ordinary paracentesis set and plastic catheters has been found to be relatively free of the hazard of infection Short-term intermittent peritoneal irrigation was described by Grollman (9) and by Legrain & Merrill (85), and modifications of this technique have been more recently reported by other authors (81, 87). Although less effective than the various forms of artificial kidney, similar results may be obtained by prolonging the period of irrigation. The technique is relatively simple and safe, and the equipment inexpensive.

The artificial kidney.—The most effective technique which presently exists for the removal of electrolyte and retained metabolites from the circulating blood is the so called "artificial kidney." The basic principle of this apparatus is the use of a cellophane membrane across which diffusible crystalloids pass from blood circulated through the membrane into a rinsing fluid on the opposite side of the cellophane. The principles of operation and design for apparatuses then extant have been reviewed in 1953 (89). Since that time an effective apparatus has been commercially produced in large volume and is in widespread use throughout the country (113, 114). Most of the artificial kidneys in general use today are effective in removing diffusible substances from the circulating blood Their efficacy depends upon the surface area of the dialyzing membrane, the rate of blood flow which may be achieved, and the skill and experience of their operators This last parameter is a far more important factor in the use of the artificial kidney than its variation in design. Where adequate facilities exist and the apparatus is operated by a trained team, the artificial kidney is an extremely important adjunct to the conservative therapy of acute renal failure Its use should not be postponed until the patient is critically ill since it may, by improving the general well-being of the patient, enable him to better tolerate conservative management Several artificial kidneys are so designed that they may be used as ultra filters (113, 115, 116) and, in such instances, the ability to remove retained edema fluid as well as metabolites constitutes a real advantage in the treatment of the overhydrated anuric patient. It should be remembered that the artificial kidney which treats acute renal failure only by correcting chemical abnormalities is effective only insofar as the morbid state is caused by this chemical abnormality and such a technique cannot be expected to be of anything but ancillary value in the treatment of surgical complications The ready commercial availability of artificial kidneys has, unfortunately, led to their use in hospitals where experience with the treatment of the whole problem of renal failure is frequently less adequate than the mechanism of the dialyzer. When correctly used, however, the efficient operation of such an apparatus adds greatly to the management of acute renal failure particularly in those instances in which augmented catabolism is a complication. Since the clinical syndrome of "uremia" results

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KIDNEY DISEASE: SURGICAL[1]

By W. L. Valk, M D. and W. S. Witus, M.D.
Section of Urology, Department of Surgery, University of Kansas Medical Center, Kansas City, Kansas

It is the purpose of this review to discuss some of the recent important advances in the diagnosis and surgical treatment of certain renal diseases. In particular, we have tried to emphasize the close relationship between the medical and surgical aspects of the various topics discussed. It is hoped that increased cooperation between the internist and the urologist will result in earlier diagnosis and treatment in many instances.

Diagnostic Techniques

Evaluation of individual renal function in hypertension.—Simultaneous collection of urine specimens from each kidney via ureteral catheters is a well-recognized diagnostic tool in the attempt to implicate unilateral renal disease as a cause of hypertension. This procedure has been described by Howard and his associates (11). The basis for the test is the observed fact that there is a decrease in urine volume and sodium concentration from ischemic kidneys in dogs The test can be performed with local or general anesthesia The patient is encouraged to drink liberal quantities of fluid in the morning before being taken to the cystoscopy room. An intravenous infusion of 5 per cent glucose in distilled water is usually given to promote diuresis. A #6 ureteral catheter is then passed to each renal pelvis and affixed to a #16 Foley catheter, which is placed in the bladder. The initial flow from each catheter should be discarded, particularly if there is hydronephrosis Urine collection should be continued until one kidney has excreted 50 cc of urine. If there is excessive hematuria incident to the catheterization, or if the flow from one or both kidneys is particularly scanty, the test should be repeated at a later date Sometimes there may be leakage of urine around the ureteral catheters If this occurs, volume measurements from each kidney will be unreliable, and the test should be repeated at another time A 15-min phenolsulfonphthalein excretion determination may be made for each kidney at the end of the initial collection period Split creatinine clearance values may also be obtained. The volume of urine from each kidney is accurately measured and the sodium concentration in each specimen determined. Howard found significantly decreased sodium concentration and total volume from kidneys whose subsequent removal resulted in improvement or cure of hypertension. To be considered significant, it is felt that the reduction in urine volume should be at least 40 per cent, and the reduction in sodium concentration at least 20 per cent

Recently, Schlegel *et al.* (47) have performed more elaborate split func-

[1] The survey of the literature pertaining to this review was concluded in August, 1959.

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clination. In patients with a non-functioning kidney, only a short OA segment is observed, with no graphic evidence of accumulation in the kidney or subsequent excretion.

Renal arteriography.—Kincaid & Davis (29, 30) have recently thoroughly reviewed the subject of abdominal aortography, including renal arteriography. The details of the technique of translumbar aortography have been well described by Kincaid (31) in another paper. Even though there is a definite calculated risk in association with this procedure (34), it is still felt to be an integral part of the examination in certain patients with hypertension in whom all other medical diagnostic studies have failed to

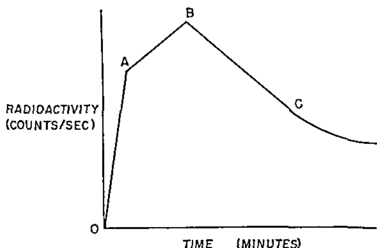


FIG 1. Diagram of a normal radioactive diodrast renogram

suggest an etiologic factor. Much of the initial work in hypertension secondary to renal artery disease has been done by Poutasse and Engel. Engel (16) has recently enumerated the indications for aortography in hypertensive patients. These include: (*a*) in young patients (under 40) with onset of recent hypertension with no obvious cause; (*b*) in older patients with acute onset of rapidly progressive or malignant hypertension; (*c*) in patients with known essential hypertension whose hypertension suddenly becomes more severe or malignant, particularly if there is associated pain of renal origin; and (*d*) patients in whom there is a significant decrease in renal size and in whom the excretory pyelogram fails to reveal the cause.

There has been some recent interest in percutaneous retrograde femoral artery catheterization for visualization of the renal arteries. Some workers feel that this procedure is safer than translumbar aortography. An opaque catheter is inserted into the femoral artery and guided up the aorta to the level of the renal arteries under fluoroscopic control. At this point, approxi-

tion studies in hypertensive patients, determining urinary sodium, potassium, ammonia, and pH from each kidney, together with inulin, *para*-aminohippurate, osmolar and urea clearances, and osmolality. In those patients in whom a definite relationship between unilateral renal disease and hypertension was proved, decreased volume, higher ammonia and potassium concentrations, higher urine osmolality, and lowered pH were found in the urine obtained from the offending kidneys.

Radioisotopes in the study of individual renal function.—The radioactive iodopyracet compound (Diodrast compound) renogram, first described by Winter (60) in 1956, is now being used as a screening test in the evaluation of individual renal function. Its particular merit is that it eliminates those patients in whom renal arteriography would probably not be of value. The test possesses several advantages (4, 60). The dose of radioiodine-tagged iodopyracet is so small (0.4 mg.), as compared to the 15 gm dose ordinarily administered for excretory pyelography, that very little attention need be paid to possible sensitization or untoward side reactions. The amount of radiation received by the patient is negligible, being approximately 1/1000 of the radiation received during an ordinary film of the urinary tract. Furthermore, the test can be performed rapidly and the results are immediately available. For these reasons, the procedure can be repeated at frequent intervals. Other advantages of the test are that no anesthesia is necessary, it can be used in place of excretory pyelography in azotemic patients in whom adequate visualization of the kidneys is unlikely, and it can replace aortography in many cases.

The test involves the intravenous injection of radioiodine-tagged iodopyracet in a dose of 1 μc per 3 kg of body weight. Two scintillation detectors, one placed over each kidney, are connected to rate meters, which in turn are connected to a graphic recording device. A graph is thus obtained which shows the rate of accumulation and excretion of the compound in each kidney The pattern of a normal radiorenogram shows three distinct stages (Fig. 1).

The three stages in a normal curve are designated OA, AB, and BC The immediate sharp spike, segment OA, represents the initial rapid rise of radioactive iodopyracet in the renal vascular pattern. The second segment of the curve, AB, represents the accumulation of the test substance in the collecting system of the kidney The downward curve, BC, reflects the excretion of the material from the upper urinary tract Because of the simultaneous uptake by the liver, the segment BC is higher in the tracing of the right kidney as compared to the left More recently, radioiodine-tagged diatrizoate sodium (Hypaque Sodium) is being used in an effort to eliminate the liver uptake factor

Typical curves have been obtained in various urologic conditions In a ... hydronephrosis, the segment BC will
... s of complete ureteral ob-
... will show an upward in-

technique have been outlined by Leadbetter, Rudy & Clarke (9) and by Chute and his associates (8). (*a*) Many tumors deemed inoperable by other approaches have been successfully removed via the thoracoabdominal route. (*b*) The approach allows early ligation of the vascular pedicle in contrast to other incisions in which extensive preliminary dissection is required before the renal vessels can be adequately exposed. (*c*) The thoracoabdominal route allows for *en bloc* removal of the tumor and contiguous structures. (*d*) The incision is adaptable in those patients with deformities of the chest wall in whom the classical approaches would be difficult. (*e*) There is a low incidence of operative mortality and morbidity.

Although many surgeons prefer to reserve the thoracoabdominal approach for large tumors of the upper pole of the kidney, Chute feels that the incision should be used in all cases of renal tumors, regardless of size or location, since local extension has been found in relatively small tumors. The operative technique, as described by Chute *et al* (8), is briefly as follows. The incision is begun posteriorly over the spinous process of the tenth or eleventh rib and is carried forward over the entire course of the rib and then extended through the abdominal wall to the lateral border of the rectus muscle The rib is then excised extraperiosteally and the pleural cavity entered anteriorly over the diaphragm to prevent accidental injury to the lung. The diaphragm is then incised and the incision carried medially into the peritoneal cavity. The latter cavity is opened widely by incising the oblique abdominal muscles and the transversus abdominus to the lateral border of the rectus. A rib retractor is then inserted and the liver or spleen protected by means of gauze packs. The duodenum or colon is then freed from the medial margin of the tumor mass. The vascular pedicle is then readily exposed and individual ligation accomplished Following this, the kidney and tumor mass, along with adjacent fascia, fat, and lymph glands are dissected free and *en bloc* removal accomplished after the ureter has been transected and ligated. Thoracotomy drainage should be utilized for at least 24 hr. postoperatively.

Renal Calculous Disease

Metabolic studies in patients with renal calculi—In approximately 95 per cent of patients with urinary calculi, the cause is unknown. One should, however, be constantly aware of possible metabolic conditions as etiologic factors in stone formation. Many patients with hyperparathyroidism have been found to have normal values for serum calcium and phosphorus or only minimal hypercalcemia and hypophosphatemia. In these cases, the urinary excretion levels of calcium and phosphorus have been found to be equivocal. The normal range for serum calcium is 9.2 to 10.4 mg./100 cc. and for serum phosphorus it is 2.5 to 4.3 mg./100 cc. Urinary calcium and phosphorus levels are determined only after the patient has been on a restricted calcium diet for at least three days. The 2000 calorie, 135 to 150 mg. calcium diet devised by Bauer and Aub is the diet used by most investigators. A normal

mately 10 cc. of contrast medium is injected and films rapidly exposed. Another technique involves the direct catheterization of each renal artery. The reader interested in the details of these techniques should refer to articles by Gregg and his co-workers (20) and by Aguzzi and his associates (1).

Renal Neoplasms

Polycythemia associated with renal tumors—Several recent reports have appeared concerning the occurrence of a secondary polycythemia in association with renal carcinoma The incidence is small, Damno *et al.* (13) observing it in 2.6 per cent of patients in a series of 350 cases of renal carcinoma. The polycythemia is usually a simple increase in total red cells without associated thrombocytosis, leukocytosis, or splenomegaly. Hematologic remission occurred in eight patients who had subsequent nephrectomy. In two cases described by Conley *et al.* (10) in which the polycythemia disappeared after nephrectomy, recurrence of the tumor was accompanied by a simultaneous reappearence of the polycythemia. The mechanism of the secondary polycythemia is unknown It has been postulated that an erythropoietic substance is produced either by the tumor itself or by surrounding normal renal tissue in response to the tumor. Another theory attributes it to a rapid shunt of arterial blood to the dilated veins of the tumor, thus stimulating increased erythropoiesis

Prognosis in renal carcinoma.—The average survival figures after nephrectomy for carcinoma of the kidney, as estimated by Riches (45) are as follows: 50 per cent of the patients will survive three years, 40 per cent will be alive after five years, and 20 per cent will live for 10 years after nephrectomy. Riches has also outlined the various factors concerned with prognosis after nephrectomy. As with the majority of other malignant neoplasms, the higher the histological grade of a renal carcinoma, the lower will be the survival rate. Involvement of the renal vein has a very adverse effect on prognosis. In a study by Thackray (56), 75 per cent of patients with grade I tumors and no involvement of the renal vein were alive five years after nephrectomy Invasion of local lymph nodes and other evidences of local extension, as well as renal vein invasion, are considered to be indications for postoperative irradiation Flocks & Kadesky (17) studied 353 cases of renal neoplasms and concluded that the results of nephrectomy combined with postoperative irradiation were better than those from nephrectomy alone Hanley (21) found that postoperative irradiation resulted in definitely better survival rates and felt that it partially counteracted the serious effect of renal vein invasion. In view of these studies, irradiation after nephrectomy should be seriously considered in the majority of patients with renal carcinoma.

The thoracoabdominal approach in surgery of kidney tumors—An important modification in the surgical approach to renal and adrenal tumors has been the use of the thoracoabdominal approach. The advantages of this

(19) found significant decreases in TRP in 21 of 22 patients with proven hyperparathyroidism. The TRP is of questionable value in the presence of azotemia because of its dependence on essentially normal creatinine clearance.

Kyle and his co-workers (33) performed a phosphate clearance test in several patients and found that it remains accurate until there is a marked fall in the glomerular filtration rate This test has also been found to be more accurate than the TRP in evaluating parathyroid deficiency states The determination is more quickly performed than the TRP and does not depend on creatinine measurements. Urine is collected over a 4-hr period and measured for phosphorus Serum phosphorus is determined midway in the collection period Phosphate clearance in cc. per minute is calculated by dividing the minute excretion of phosphorus expressed in mg. by the serum phosphorus in mg./cc. The mean value in 25 normal persons was found to be 10 8 plus or minus 2 7 cc./min The value is significantly elevated in hyperparathyroidism and decreased in parathyroid deficiency.

It must be emphasized that in the majority of patients with hyperparathyroidism, the ordinary diagnostic procedures will usually suffice to make the diagnosis. Where these values are equivocal and clinical suspicion of parathyroid hyperfunction remains high, a combination of the above tests should be performed.

Keating (28) has recently emphasized the fact that the majority of patients with renal stones exhibit no well-defined abnormalities in calcium metabolism He then described the various metabolic disease states in which calculi are formed These include, in addition to primary hyperparathyroidism, hypervitaminosis D, multiple myeloma, sarcoidosis, milk-alkali syndrome, osteoporosis of disuse, Paget's Disease, and metastatic carcinoma involving bony structures Hypercalcemia is present in all of these conditions, and the clinical symptoms of all may be strikingly similar.

A recent report by Henneman and his associates (24) is of interest. They describe a syndrome consisting of hypercalciuria, hypophosphatemia, and normal levels of serum calcium in association with renal calculous disease. Exhaustive study in a group of 24 patients failed to reveal a definite metabolic defect This condition has been referred to as idiopathic hypercalciuria. In several of these patients, exhaustive surgical search for a parathyroid adenoma was carried out with negative results. The authors propose a possible chain of events to account for the syndrome Initial pyelonephritis leads to tubular damage with resultant decreased reabsorption of calcium from the glomerular filtrate This, in turn, leads to increased amounts of calcium in the urine and a decrease in the serum calcium level Parathyroid hyperplasia ensues as a compensatory mechanism leading to an increase in urinary phosphorus and consequent decrease in serum phosphorus levels Certain patients with similar laboratory findings may have a congenital tubular defect in which base conservation is impaired Calcium is wasted,

person on this diet for three days will excrete 100 mg. or less of calcium in the urine per day. Values above 150 to 200 mg. per day are considered abnormal.

Because of equivocal results obtained from the usual laboratory procedures in several patients who subsequently proved to have hyperparathyroidism, several new diagnostic tests have been devised. (*a*) In the phosphate deprivation test the patient is placed on high-calcium, low-phosphorus diet for a short period of time, usually one to two weeks. Repeat laboratory data after that period will either show more definite evidence of hyperparathyroidism, or else previous equivocal data will have returned to normal values (*b*) The calcium loading test (6) measures changes in serum and urinary phosphorus after the infusion of large amounts of calcium. Fifteen to 25 mg. of calcium/kg., as calcium gluconate, is given as an intravenous infusion over an 8-hr. period. Blood phosphorus levels are determined prior to starting the test, at the midpoint, and at the end of the infusion period. Twenty-four-hour urine specimens are collected on the day before the test is performed and on the day of the infusion and then are measured for total phosphorus content. A normal individual will exhibit a 30 to 50 per cent decrease in urinary phosphorus and a significant elevation of serum phosphorus over levels determined prior to the infusion. A patient with hyperparathyroidism will not exhibit these changes. (*c*) Perhaps the most popular of this group of tests is the phosphate reabsorption test or tubular reabsorption of phosphate (TRP) (19). In the normal person, the renal glomeruli filter approximately 5000 mg. of phosphorus per day. Of the filtered phosphorus, 80 to 90 per cent is normally reabsorbed, resulting in a urinary phosphorus excretion of about 750 mg per day In hyperparathyroidism, the excessive hormone produces an increase in urinary phorphorus excretion because of increased inhibition of phosphorus reabsorption. Thus, the percentage of reabsorbed phosphorus is considerably reduced in these patients

The clinical determination of TRP involves the simultaneous determination of serum and urinary creatinine and phosphorus levels during a fixed period of time. Endogenous creatinine clearance is calculated from the formula UV/P where U equals the urinary concentration of creatinine in mg./100 cc., V equals the urine volume in cc./min., and P equals the plasma concentration in mg./100 cc. The resultant creatinine clearance, in cc./min., multiplied by the serum phosphorus concentration, will give the amount of phosphorus filtered per minute. The amount of phosphorus reabsorbed by the renal tubules is calculated by substracting the amount of phosphorus excreted in the urine per minute from the amount filtered per minute. The amount reabsorbed divided by the amount filtered, multiplied by 100, results in the tubular reabsorption of phosphorus expressed as a percentage. The normal range of TRP in 50 normal persons was found by McIntosh *et al* (36) to be 84 to 97 per cent TRP values ranging from 71 to 80 per cent were found in five patients with hyperparathyroidism by the same worker. Goldman *et al.*

identical twins, since a transplant from an unrelated donor will ultimately fail because of antibody formation in the recipient. Work is presently being done in an attempt to negate the antibody response, permitting successful transplantation of a donor kidney to an unrelated recipient. Transplantation was carried out in seven monozygotic twins, four of whom had chronic glomerulonephritis, two had chronic pyelonephritis, and one patient had subacute glomerulonephritis. Successful results up to the time of publication of the article were obtained in six of these patients, with the return of renal function to normal.

The authors emphasize that the most important factor in transplantation is the health of the donor. He or she must have completely normal renal function and a normal lower urinary tract without evidence of urinary tract infection. The lower urinary tract in the recipient must also be normal before transplantation is considered. The uremia must be irreversible, and the causative renal disease must be in an inactive state. Cross skin grafts are performed initially and observed for three weeks to prove that the twins are monozygotic. The technique presently utilized involves placing the kidney in the iliac fossa on the opposite side from which it was removed End-to-end anastomoses between the renal and internal iliac arteries and the renal and external iliac veins are accomplished, followed by a ureterovesical anastomosis In successful transplantations, urine flow in large volumes begins almost immediately As soon as possible after successful transplantation has been accomplished, the diseased kidneys are removed to prevent reinfection or hypertension

Ureteroileostomy.—Perhaps one of the most important recent advances in urologic surgery has been in the field of urinary diversion. The various indications for diversion of the urine have been outlined by Creevy (12). They include (*a*) after cystectomy for carcinoma of the bladder or in pelvic exenteration performed for other neoplasms; (*b*) malfunction of the bladder secondary to neurogenic disease, irreparable vesical fistulae, or exstrophy of the bladder; (*c*) to arrest or improve renal damage secondary to previous ureterosigmoidostomy; (*d*) in severe vesical contracture as sometimes occurs in tuberculosis and interstitial cystitis The multitude of diversionary procedures is evidence of the fact that no perfect operation has been devised to date. Some of the presently used methods are nephrostomy, pyelostomy, cutaneous ureterostomy, "wet" colostomy, and ureterosigmoidostomy.

In 1954, Bricker (5) described a method of urinary diversion utilizing an isolated segment of ileum which has been variously referred to as ileocutaneous ureterostomy, ureteroileostomy, ileal conduit, or the Bricker operation. This procedure is being utilized with increasing frequency by many surgeons and promises to eliminate many of the shortcomings inherent in other diversionary operations. Perhaps the most important factor in urinary diversion is the preservation of renal function, and ureteroileostomy

leading to hypercalciuria and subsequent stone formation. These patients may also exhibit a hyperchloremic acidosis.

Renal complications in the treatment of leukemia.—The urologist is occasionally called upon to aid in the treatment of a patient with leukemia who rapidly develops azotemia or anuria after the institution of x-ray or drug therapy. In these cases, the marked increase in uric acid excretion has obstructed the renal tubules, pelvis, or ureter with soft urate crystals. McCrea (35) has pointed out that the incidence of uric acid renal calculi in patients with leukemia is approximately 5 per cent, as contrasted to the calculated incidence of 0 07 per cent in the general hospital population This difference is attributable to the known increased levels of serum and urine uric acid in leukemic patients, which are often markedly accentuated shortly after the institution of treatment. This is seen particularly in acute lymphocytic and acute and chronic myelogenous leukemia. Increased purine catabolism, of which uric acid is a major product, results from the destruction of nucleoproteins as a result of therapy. Kritzler (32) reported three cases of anuria caused by uric acid obstruction of the urinary passages complicating leukemia. He also reviewed seven cases previously reported in the literature. Obstruction of the renal pelvis or ureter was demonstrated in eight of these patients In the other two patients, it was assumed that anuria was caused by uric acid obstruction of the collecting tubules

Certain precautionary measures should be carried out in all leukemic patients about to undergo therapy A pretreatment excretory urogram is useful to rule out the possibility of silent urinary tract obstruction secondary to previously formed uric acid calculi The concentration of urea or uric acid in the serum should be determined prior to treatment and these tests should be repeated at frequent intervals A high fluid intake of at least 3000 cc per day, should be maintained in an effort to reduce the urinary urate concentration. Alkalinization of the urine by the administration of sodium bicarbonate or sodium citrate is also advisable since the solubility of uric acid is increased in an alkaline urine All of these measures should be utilized in patients with gout who are receiving long-term therapy with uricosuric agents, such as probenecid

A rising concentration of serum uric acid or urea should suggest possible cessation of therapy and, if oliguria or anuria occurs, bilateral ureteral catheterization should be carried out and the catheters left indwelling for several days The catheters should be irrigated at frequent intervals with saturated alkaline solutions. Occasionally, nephrostomy is required to achieve adequate urinary drainage.

Advances in Surgical Technique

Renal transplantation.—Murray and his associates (39) have recently summarized their further experiences with kidney transplantation. At the present time, permanent function from a transplanted kidney occurs only in

pressure and constant admixture of urine and feces is avoided in the Bricker operation. It should be mentioned that the reference to the isolated segment as an "ileal bladder" is a misnomer. When properly performed, there is no stasis of urine in the segment and peristalsis of the ileum continuously propels urine into the collection device. Thus, the isolated ileal segment serves as a conduit rather than a reservoir. Whereas ureterosigmoidostomy is generally contraindicated in the presence of significant ureteral dilatation, ureteroileostomy can be performed safely when this condition exists Further long-term follow-up studies are required before complete evaluation of ureteroileostomy can be made, but it is the opinion of many urologists that the procedure is as close to the ideal method of urinary diversion as has yet been devised.

Splenorenal arterial anastomosis.—Seidenberg and his associates (26, 49) removed the right kidney and spleen from dogs and then anastomosed the splenic artery to the left renal artery (27, 50). They found that the splenic artery furnished enough blood to the kidney to preserve renal function. The authors enumerate several instances wherein this procedure may be utilized Splenorenal arterial anastomosis may be performed in cases of unilateral renal arterial disease in place of nephrectomy, thromboendarterectomy, or homograft replacement of the diseased segment of the renal artery A case is reported in which the operation was successfully performed in the presence of bilateral renal artery obstruction secondary to aortic thrombosis Its use has also been suggested in the surgical treatment of abdominal aortic aneurysm to avoid the dangers of bilateral renal artery obstruction The authors have also utilized the inferior mesenteric artery as a substitute renal artery In a case of bilateral renal artery stenosis, as reported by Poutasse (43), in which stenotic segments of each renal artery were replaced by homografts, the author suggests that the splenic artery could have been used on the left side and the inferior mesenteric artery utilized on the right side to by-pass the obstruction

Renal hypothermia—Some interesting work has been done in the study of the effects of regional renal hypothermia on kidney function Van Slyke *et al* (58) occluded the renal artery for variable periods of time in uninephrectomized dogs If the artery was occluded for a period of 3 hr. or less, eventual return of normal renal function resulted. Fifty per cent of the dogs died if the artery was occluded for over 3 hr, and all dogs died if the artery was clamped for 6 hr Hardin & Valk (23) performed similar experiments in a group of dogs in which one kidney had previously been removed They found that the critical period after cross-clamping of the remaining renal artery was 2 hr, and that variable degrees of impaired function resulted when renal ischemia of longer duration was produced Both of the above studies were carried out under normothermic conditions. Moyer *et al* (37) studied the effects of total body hypothermia on renal function in dogs after

appears to have accomplished this in the majority of cases followed to date. Progressive renal deterioration often occurs after nephrostomy, cutaneous ureterostomy, and ureterosigmoidostomy.

A brief description of the technique is worthwhile at this point. A vertical midline incision is used, extending from above the umbilicus to the symphysis. The ureters are then isolated low in the pelvis and transected. The ureters are then dissected upward, care being taken to preserve the vascular supply. The left ureter is dissected up much higher than the right since it must be tunneled through the sigmoid mesentery. This is accomplished by bringing it through an avascular area of the mesentery so that it lies within the peritoneal cavity. The incisions made in the posterior peritoneum are then closed. An appendectomy is carried out, and a segment of ileum close to the ileovesical junction is isolated. This segment should preferably be supplied by two vascular arcades. The length of the ileal segment should be no longer than 10 to 12 cm since an excessively long loop may cause urinary stasis and subsequent excessive electrolyte absorption. The ileal segment is then isolated and the continuity of the ileum re-established by end-to-end anastamosis. The segment is brought in front of the ileoileostomy and the mesentery of the segment is closed. The proximal end of the segment is closed in an inverted fashion and the open distal end brought through the abdominal wall at a point midway between the umbilicus and the anterior superior iliac spine on the right side. A circular portion of the entire abdominal wall is excised at this point to prevent subsequent contraction of the ileal stoma by scar tissue. The end of the ileum is sutured to the abdominal wall, producing a protruding bud of ileum. Each ureter is then anastomosed to the antimesenteric border of the ileal segment, using a single layer of interrupted fine chromic sutures through all layers of the ureter and ileum. At the completion of the operation the ileal stoma should admit one's finger with ease. A temporary plastic bag or permanent Rutzen-type of collection device is placed over the ileal stoma for collection of urine. We have found that after patients have learned to care for and apply these bags, they may be kept on for as long as five to seven days without changing.

This procedure is not without its disadvantages. First and foremost is that the patient sacrifices urinary continence and is forced to wear a collection device. The operation takes much longer to perform than most other methods of diversion and there is a greater incidence of postoperative complications, the most frequent being ileus. The procedure, however, has many advantages. Studies to date have indicated that electrolyte resorption has not been a problem in ureteroileostomy. This is in contradistinction to ureterosigmoidostomy in which more than 50 per cent of patients develop hyperchloremic acidosis. In addition, patients with ureterosigmoidostomy have a high incidence of ascending urinary tract infection and pyelonephritis with resulting progressive deterioration of renal function. The high intracolonic

method with regard to healing and preservation of function in the remaining renal tissue (39). They performed a partial nephrectomy in three groups of dogs, utilizing a different technique in each group. In one group a wedge type of resection was done with closure of the remaining parenchyma by means of mattress sutures. A transverse resection was carried out in the second group and the raw surface of the kidney was covered by a free peritoneal graft In the third group, a transverse resection was performed but, instead of a peritoneal graft, the renal capsule was loosely approximated over the raw surface. Rates of healing and the amount of damage to remaining parenchyma were then compared. Optimum healing with minimal renal destruction occurred with the method used in the third group of dogs. The authors successfully applied this procedure in five patients with excellent results The observations made by Murphy and Best will undoubtedly encourage more urologists to perform partial nephrectomy in conditions where nephrectomy has been commonly utilized

Urologic Aspects of Hypertension

Approximately five per cent of hypertensive patients exhibit unilateral or bilateral renal disease which could possibly be responsible for the elevated blood pressure (40). In only a small percentage of these patients is the disease truly unilateral Experience to date indicates that nephrectomy will produce a "cure" of hypertension in approximately 20 to 25 per cent of patients in whom unilateral renal disease is thought to be the etiologic factor.

Engle (16) has outlined the various pathologic conditions of the kidney which may be associated with hypertension These include infections, hydronephrosis, calculous disease, congenital anomalies, tumors, and renal artery disease producing ischemia. Pyelonephritis, tuberculosis, perinephritis, and pyonephrosis are some of the types of renal infection which can cause an elevation in blood pressure Polycystic disease is an example of a congenital renal condition which may be associated with hypertension. Many of these conditions are often bilateral and thus nephrectomy cannot be considered. According to Thompson (57), much better results are obtained from nephrectomy in patients with unilateral atrophic pyelonephritis than with other lesions (59). Fifty per cent of patients with unilateral atrophic pyelonephritis are benefited by nephrectomy, in contrast to the estimated 25 per cent who gain relief when their hypertension is associated with other types of renal lesions

The various types of renal artery abnormalities which may produce hypertension have been described by Poutasse (43) and by Glazier & Lombardo (18). Degenerative changes in the renal artery may produce renal ischemia and thus initiate the humoral mechanism thought to be responsible for the elevation in blood pressure. Arteriosclerotic changes in the

occlusion of the renal vascular pedicle for 1 to 3 hr. They found that generalized hypothermia served partially to protect the kidney from the effects of ischemia. Since the production of generalized body hypothermia poses some dangers, Stueber and his associates (53, 54) have recently reported on their experiments utilizing regional renal hypothermia (55, 56). The right kidney was removed in three groups of dogs, ten in each group Two weeks later, the vascular pedicle of the left kidney was clamped for a 6-hr. period The procedure was carried out under normothermic conditions in a control series of ten dogs, and all ten died within a period of five days. Widespread coagulation necrosis was observed in the proximal convoluted tubules in the kidneys of these dogs. In the second group, the kidneys were cooled by means of a plastic jacket to temperatures of 20 to 25°C. during the period of ischemia. Three of the ten dogs survived under these conditions. In the third group, cooling was accomplished to temperatures of 10 to 15°C. and in this group seven dogs survived. Increased concentration of urea in the blood was significantly less in the group in which the temperature had been lowered to 10 to 15°C. The possible clinical applications of regional renal cooling are numerous. In surgical replacement of an abdominal aortic aneurysm with renal artery involvement, regional cooling of the kidney may serve to prevent renal damage during the temporary period of ischemia. In partial nephrectomy, nephrolithotomy, and certain other surgical procedures on the kidney, temporary occlusion of the renal artery is often produced either by finger compression or a bulldog clamp. In this manner, bleeding during the procedure is minimized. It is possible that renal function may be protected from the effects of the temporary ischemia by a preliminary period of cooling of the kidney.

Surgery in renal tuberculosis.—Prior to the advent of specific chemotherapeutic agents, nephrectomy was commonly performed for tuberculous renal lesions. Modern drug therapy has eliminated the need for nephrectomy in the overwhelming majority of patients with renal tuberculosis Hanley (22) has recently summarized the present-day indication for surgical treatment in renal tuberculosis The major indication now is corrective surgery for the obstruction produced in the urinary tract by the healing fibrosis. Progressive fibrosis at the ureteropelvic junction may lead to pyohydronephrosis and, in Hanley's experience, has been the chief indication for nephrectomy. Healing in the infundibular area of the collecting system may lead to obstruction of a calyx, producing a pyocalyx. The treatment of such a complication is often partial nephrectomy. Stricture formation incident to healing of tuberculous ureteritis sometimes occurs at the ureterovesical junction, necessitating ureteroneocystostomy or nephroureterectomy, depending on the functional status of the kidney.

Partial nephrectomy—Murphy & Best (38) have recently reviewed the methods of partial resection of the kidney in an effort to determine the best

Typical evidence of pyelonephritis may be seen. In most cases of renal artery disease, however, the affected kidney appears perfectly normal on excretory pyelography. Retrograde pyelography should be performed when indicated.

Scott (48) has suggested the following screening program after the above studies have been completed. The radioactive iodopyracet renogram should be performed initially. If the renogram shows a decreased blood flow to one kidney, split renal function studies as outlined by Howard and Schlegel should be carried out If the latter tests are equivocal, renal arteriography should be done. In cases where the excretory urogram demonstrates a definite unilateral abnormality such as atrophic pyelonephritis, the patient need not be subjected to aortography. In such cases the Howard test should be performed, followed by nephrectomy if indicated.

Until the last three or four years, the only surgical treatment in cases of hypertension associated with unilateral renal disease has been nephrectomy. With improvement in techniques of vascular surgery, several ingenious operations have been performed with excellent results in patients whose hypertension was secondary to disease of the renal artery. Humphries & Poutasse (25) have utilized a segment of donor aorta, including the renal arteries, to replace a diseased renal artery. Others have used segments of the femoral or iliac artery for this purpose. In cases where the abnormality is close to the origin of the renal artery from the aorta, the artery may be transected and the distal normal segment re-implanted into the aorta DeCamp and his associates (15) reported a case of a 10-year-old girl with marked hypertension secondary to a poststenotic aneurysm of the left renal artery. The right kidney was congenitally absent. Surgical treatment consisted of splenectomy and end-to-side anastomosis of the splenic artery to the dilated portion of the renal artery. Postoperatively, the blood pressure fell to normotensive levels and continued to be normal approximately a year later. A similar procedure was carried out by Parton & Nabseth (41) in a 61-year-old man in whom an arteriosclerotic plaque was found to partially occlude the opening of the left renal artery. We have mentioned earlier in this review that the splenic artery, as well as the inferior mesenteric artery, has been successfully substituted for the right renal artery in dogs. To date, replacement of the right renal artery by the splenic or inferior mesenteric has not been reported in human beings. Other operative procedures which have been performed in renal arterial disease include endarterectomy and excision of the diseased segment with re-anastomosis If only one segment of the renal arterial tree is diseased, producing an infarction of one pole of the kidney, heminephrectomy instead of total removal, has been successfully performed In all of the above procedures, regional renal hypothermia may be found to be of value to prevent any deleterious effects which may result from prolonged interruption of blood flow to the kidney.

renal artery may be complicated by thrombosis with complete interruption of renal blood flow resulting in renal infarction and acute hypertension Bryant and his associates (7) have described a case in which a 68-year-old man experienced a sudden attack of abdominal and right costovertebral angle pain Excretory pyelography revealed nonvisualization of the right kidney and a normal left kidney. Retrograde pyelography demonstrated normal architecture of the right kidney. No urine was seen to be coming from the right kidney. The blood pressure was markedly elevated Subsequent exploration of the right kidney revealed infarction secondary to renal artery thrombosis The blood pressure fell to normal levels after nephrectomy and remained at normotensive levels after one year.

Renal ischemia may also result from renal artery embolism or by extrinsic occlusion of the artery produced by a retroperitoneal tumor. The various congenital anomalies of the renal artery which have been reported to produce hypertension are coarctation, unilateral atresia, cirsoid angioma, and aneurysm.

In general, authorities agree on the type of patient who should undergo intensive study in an effort to rule out a unilateral renal lesion as a basis for hypertension. Young patients (under 35 or 40) with elevated blood pressure of two years or less duration will probably be the most benefited by *surgical correction of unilateral renal disease* DeCamp & Birchall (14) point out the fact that it is the opposite kidney which is exposed to the hypertension and which may suffer from irreversible vascular changes if the causative lesion remains undiscovered for a long period of time. Patients in any age group with benign essential hypertension who suddenly develop malignant hypertension should be thoroughly studied Any patient, regardless of age, previously known to be normotensive, who suddenly develops severe or malignant hypertension, should be suspected of having unilateral renal pathology. This is particularly true if the hypertension has been ushered in by an attack of flank pain.

Initial study should consist of a laboratory evaluation of total renal function including blood urea, creatinine, phenolsulfonphthalein excretion, and urea or creatinine clearance. Further studies may not be indicated if these studies indicate a significant decrease in total renal function, since bilateral renal disease then is present Even if the original cause of the hypertension were unilateral renal disease, the opposite kidney has suffered damage from vascular changes and removal of the initially involved kidney might actually shorten the patient's life span in addition to having no effect on the hypertension. Excretory pyelography should then be performed and any difference in the size of the renal shadows on the film should be carefully noted. The intravenous pyelogram may show delayed function, poor concentration of contrast medium, or total non-visualization of one kidney. Kidney architecture may be altered by numerous pathological conditions

tis and tabulated the significant clinical findings (53). Sixty-two per cent of the patients had albuminuria, and pyuria was demonstrated in 58 per cent. Thus, in a significant number of patients, the classical laboratory findings were absent. Because of this fact, Kass has emphasized that the demonstration of significant numbers of bacteria in the urine should be the primary focus in the diagnosis of pyelonephritis Present studies indicate that active pyelonephritis is present when the count reveals 100,000 or more bacteria per cc. of urine.

Rattner *et al.* (44) have attempted to show the method by which bacteria reach the blood streams from renal infections (46). A polyethylene tube was inserted through the thoracic duct into the cisterna chyli and one ureter clamped. A ureteral catheter was then inserted to the kidney pelvis on the opposite side. Control samples of lymph were obtained. A bacterial suspension was then injected through the ureteral catheter into the renal pelvis, the catheter removed, and the ureter ligated. Serial lymph samples were obtained together with excision of lymph nodes between the renal pelvis and the cisterna chyli The nodes and lymph specimens were found to be positive for the inoculated organisms. The study indicated that pathogenic organisms from infected kidneys enter the blood stream by way of renal lympatics

Much of the recent experimental work in pyelonephritis has been done by Beeson and his co-workers at Yale University (46). In one group of experiments, renal lesions were induced by means of an electric cautery followed by intravenous injections of bacterial suspensions. Subsequent studies revealed that the medullary area was much more susceptible to infection than the cortex. The reason for this difference is thought to be obstruction in the collecting tubules In another group of animals with similarly induced lesions, bacterial suspensions were introduced into the bladder. Pyelonephritis was evident in the region of injured medulla in 9 of 20 animals.

Weyrauch *et al.* (59) have studied the effect of prophylactic antibiotics in experimentally induced pyelonephritis. Experimental infection was produced in rabbits by the intravenous injection of *E. coli* organisms 24 hr. after the partial ligation of one ureter. Tetracycline administered before ligation was found to prevent or significantly lessen infection in comparison to those animals which did not receive the antibiotic.

There have been many reports in the literature incriminating the urethral catheter as an important factor in the etiology of pyelonephritis. That bacteriuria accompanies the use of an indwelling catheter in the majority of cases is an undisputed fact. Kass found significant bacteriuria in 95 per cent of patients three to four days after the institution of indwelling catheter drainage. However, many authors have disputed the relationship of this bacteriuria to subsequent pyelonephritis. Prather & Sears (42) have stated that the most important factor is whether or not

Pyelonephritis

Many recent articles have appeared concerning the pathogenesis, diagnosis, treatment, and prevention of pyelonephritis. It is the most common of all renal diseases. According to Kass (27), it is found in 10 to 20 per cent of kidneys examined at autopsy, and in a significant number of these patients the disease was not diagnosed during life. Many patients who are found to have chronic pyelonephritis will give no history suggestive of previous acute urinary tract infections. Kass and others have emphasized the extreme clinical importance of chronic pyelonephritis (2, 27). It is known to be much more common in the diabetic than the non-diabetic. The factors in diabetes which contribute to urinary tract infection are vascular disease, a general increased susceptibility to all types of infection, the effect of glycosuria on bacterial growth, and an increased number of catheterizations incident to treatment of diabetic complications Pyelonephritis has been implicated in the etiology of both essential and malignant hypertension and it is the most common kidney lesion found in uremia.

Beeson (2) has summarized the various factors in the pathogenesis of pyelonephritis in an excellent article. He discusses at length the three possible routes by which bacteria may reach the kidney. One theory is that bacteria ascend to the kidney from the lower urinary tract via the lymphatics. However, as Beeson points out, there is no conclusive evidence that the upper and lower urinary tracts have lymphatic connections Hematogenous spread is the accepted pathway by which coccal infections reach the kidney. Furthermore, bacteremia is definitely known to occur in many instances during and after various endoscopic procedures on the bladder and urethra There is doubt, however, that this is the most common pathway by which bacteria reach the kidney. Ascending infection via the ureter is probably the most common route by which bacteria travel to the upper urinary tract Talbot (55) feels that the ureter plays a dual role in the pathogenesis of pyelonephritis (58) First, infection may spread by way of the subepithelial tissue of the ureter which is in direct continuity with the bladder mucosa and the renal pelvis Second, anatomic changes in the ureteral wall incident to subepithelial inflammation may result in a functional obstruction to the column of urine, altering ureteral peristalsis and producing stasis of the urine. This can cause the same type of obstruction as would be seen in cases of ureteral calculus, extrinsic compression of the ureter, or vesical outlet compromise. Furthermore, the high incidence of vesicoureteral regurgitation in patients with chronic lower urinary tract obstruction and infection is attributed to the fact that inflammatory changes in he ureteral wall, particularly in the intravesical portion of the ureter, interferes with the normal valve-like action at the ureterovesical junction. It is this latter mechanism which is thought to prevent the reflux of urine from the bladder in the normal person.

Smith & Lenyo (51) studied 100 patients with proven active pyelonephri-

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the urinary infection remains after the catheter is removed. In defense of the use of the urethral catheter, Prather states,

We find no evidence to implicate the catheter as a significant factor in the cause of chronic pyelonephritis No doubt the majority of patients who develop acute pyelonephritis have never been catheterized prior to the onset of the acute infection. It is not logical to blame the catheter as a major contributor to serious urinary tract infections (unless it has been used improperly) simply because the instrument has been used as a diagnostic or therapeutic agent in patients with urinary tract disease. The catheter, properly used, is one of man's best friends (45).

There can be no doubt that when serious urinary tract infection has occurred following catheterization, unrecognized urinary tract pathology has often been present or poor catheter technique has been used.

ENDOCRINOLOGY: THE THYROID[1,2]

By Russell Fraser, F.R.C.P.
Department of Medicine, Postgraduate Medical School of London, London, England

Of the extensive recent experimental work on the thyroid this review will only be concerned with lymphadenoid goitre, the control of thyroid function, the carriage of the hormone in the blood, and the action of the hormone on the tissues. Much of the current knowledge on the thyroid hormone has been reviewed in a recent Ciba Symposium (61), and in Pitt-Rivers' & Tata's book, *The Thyroid Hormones* (111).

Lymphadenoid Goitre

A major recent development has been the discovery that autoimmunity is the basis of lymphadenoid goitre or Hashimoto's thyroiditis (80, 102). Studies moved from two directions: first, for many years thyroglobulin seemed to be a suitable antigen for studying autoimmunity (160); on the other hand, the puzzle of abnormal "liver function tests" as found in the plasma of patients with Hashimoto's disease and myxedema, led Roitt *et al.* (124) to find in these sera antibodies to thyroid extracts, prompting their suggestion that an autoimmune reaction was the basis of the disease.

The earliest produced precipitins to thyroglobulin had been weakly organ-specific. However, when Freund's adjuvant was added to the injections, an animal could be made to develop precipitins to its own thyroglobulin which were largely species-specific (158, 159); and its remaining thyroid lobe was found to show Hashimoto-like lesions. In comparison, the patients with Hashimoto's disease appear to be hyperimmunised; as much as 5 mg per ml. of antibody may be found in their serum, an excess of what can be produced by injecting an antigen (125). Within two or three years after thyroidectomy, the titre of these antibodies falls off

The antibody has also been demonstrated in thyroid slices by fluorescent techniques: in the follicular colloid and cells, and also interstitially in the regions of collections of plasma and lymphocyte collections (156)

The precipitin test on serum may reveal only the higher titres of the thyroglobulin antibody, but lesser amounts can be revealed by the sensitive tanned red cell haemagglutination test. This, and the other known antibodies found in these sera are γ-globulins, which fraction is usually found

[1] The survey of the literature pertaining to this review was concluded in May, 1959

[2] The following abbreviations are used BMR (basal metabolism rate); PBI (protein or peptide-bound iodine); TBP (thyroxine-binding protein); TBC thyroxine-binding capacity); T/S ratio (thyroid to serum iodide gradient); TSH (thyroid-stimulating hormone).

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iodine to thyroxine (32). Thyroid therapy is usually sufficient to suppress the process; indeed, McConahey *et al.* (86) suggested that most non-toxic goitres responding to such therapy are really lymphadenoid goitres. It remains to be seen whether this therapy is enough, for it may be important to remove the source of autoimmunisation

Thyrotropin Secretion

Hypothalamic control—This control is probably mediated by the hypophyseal portal system whose blood flows from the median eminence to the anterior pituitary (72). Greer (61) has reviewed much of his own and other work relating to the control of thyrotropin secretion The pituitary transplanted to the eye of an hypophysectomised host maintains a rate of thyroid secretion between that of the normal and hypophysectomised animal without, however, increasing its thyroid weight Furthermore, its thyrotropin secretion can still be weakly stimulated by propyl thiouracil or depressed by thyroxine administration, i e, it is still subject to the thyroxine "feedback" control. The same alterations in thyrotropin secretion can also be produced by stalk section or hypothalamic lesions involving the region of the median eminence Yet, electrical stimulation of this region does not increase thyrotropin secretion of thyroid activity. These animals, without pituitary-hypothalamus contact show, on propyl thiouracil, little or no goitrogenesis but maximally increased thyroid avidity for iodide (iodide gradient, or T/S ratio) Greer postulated that there were two thyrotropins, a "growth" factor and a "metabolic" factor, the former needing the connection with the hypothalamus However, on goitrogen-treated rats maintained at several graded levels of thyroxine dosage, Bogdanove (20) found that slight thyroxine deficiency raised the T/S ratio to maximal levels before any goitre developed, while greater thyroxine deficiency produced goitres but no further increases in the T/S ratio Clearly, the iodide-trapping efficiency is a function of avidity × size. Pretty experiments by Purves (113) have offered an explanation for the mechanism of this hypothalamic control His [illegible]

creased rate of thyroid secretion which either low iodine diet, propyl thiouracil, or thyroxine administration could still modify in the normal directions When they were given, in addition to propyl thiouracil, graded doses of thyroxine, maximal suppression was observed with a third of the thyroxine dosage needed to achieve this in normals Thus, the thyroxine-sensitive centre is probably in the anterior pituitary itself; and the hypothalamus modifies this control by its own preliminary removal or inactivation of the thyroid hormone from the blood passing through it to the anterior pituitary

This theory explains the observed effects of these lesions on thyroid

raised to levels beyond those expected of these known antibodies The erythrocyte sedimentation rate also is always raised. These antibody changes are greatest with large glands and are unrelated to thyroid function; but they are indications of the disease rather than its cause (*vide infra*). As many as three separate thyroglobulin antibodies are demonstrable in these sera, corresponding probably to the several thyroglobulins found in the thyroid (125). Another and different antibody has also been demonstrated in the serum of these patients, a "thyrotoxic complement-fixing" antibody✓ whose antigen is obtained in highest yields from hyperplastic thyroid tissue (from thyrotoxic or dysgenetic goitres). This antigen is found not in the thyroglobulin but in the microsomal fraction, so that it is probably a cell protein (8, 126) By using sensitive techniques, both of these antibodies have been shown in high titre in nearly all (over 90 per cent) sera from cases of active Hashimoto's thyroiditis, and in moderate titre almost as frequently in spontaneous myxedema cases Often (50 to 70 per cent), moderate or low titres have been found in sera from thyrotoxicosis and subacute thyroiditis, and low titres much less frequently in most thyroid diseases (8, 59, 103, 104, 126). There is also a small amount to be found in elderly normal subjects in a frequency rising with age (59), which may correspond to the similar finding for Askanazy cells in routine postmortem thyroids (82).

Stuart & Allan (144) have demonstrated what may be the essential lesion for this autoimmune reaction in the thyroid, i e., focal defects in the basement membrane of the thyroid follicle, whose incidence was found to correlate well with the presence of antibodies in the serum However, injections of Hashimoto's serum have damaged neither monkeys' thyroid *in vivo* nor cultures of human thyroid tissue (126) Thus, it is probable that the antibodies in the serum are not the cytotoxic factors; these may be antibodies acting against some cell-surface antigens, possibly produced locally by the lymphocytes What initiates this cytotoxic process remains a problem

Needle biopsy reveals the disease in several apparently simple non-toxic goitres Histological diagnosis is based on the finding of infiltration with lymphocytes and plasma cells, and the Askanazy eosinophilic degeneration of follicular cells; the latter may imply failure of the cells under maximal stimulus (14, 162) When the process is only seen focally it has a different significance, as does a low titre of antibodies in the serum

The diagnosis is better confirmed by high titres of antibodies or γ-globulins in the serum, than by ^{131}I tests; the needle biopsy will settle doubtful cases ✓ There are several studies of the goitre's use of ^{131}I. Thyroid uptake of ^{131}I may be varied but there is always a rapid discharge which can be accelerated by perchlorate at 1 hr, and the glands do not respond to TSH [suggesting that they are under maximal stimulation (32, 92)]. Owen & McConahey (101) found an unusual iodinated protein in the serum of some cases. These tests indicate both defective storage and incomplete synthesis of the hormone. One hydrolysed gland contained less than half the usual ratio of

ments, that ACTH may act synergistically with TSH to produce exophthalmos. Further data from patients are, however, needed, and the relation of TSH to thyrotoxicosis still remains an unsettled problem, as does the cause of exophthalmos (3), in which the pituitary at least plays some part. Adding to previous reports of benefit to exophthalmos from cauterization or surgical removal of the pituitary, is another following yttrium implantation (91).

Hormonal Biosynthesis and Goitrogenesis

The transformation of iodide to secreted hormone by the thyroid has been reviewed (111, 122), the main steps of which are: (*a*) the concentration of iodide into the gland: (*b*) the organic binding of this to mono- and diiodotyrosines; (*c*) the coupling of these to form the thyronines, of which the major portion is thyroxine; (*d*) final hydrolysis of the stored thyroglobulin to release the thyronines into the blood, while simultaneously (*e*) a dehalogenase returns most of the tyrosine iodine back for further synthesis The enzymes for the first four reactions have yet to be defined, as has also the importance of various allied compounds formed in smaller amounts.

Goitrous cretins—Severe congenital derangements in this biosynthesis cause hypothyroidism together with a goitre Many of these cases have now been studied by ^{131}I procedures, designed to reveal which of the above major steps are defective [reviewed in (137) and (140), with a tabulation in (81)]; defects at each of the last four steps have been found. The defect at step (*b*) is easily recognised by finding an avid thyroid uptake which is discharged by thiocyanate or perchlorate (127, 134); that at step (*d*) from the release into the circulation of protein- or peptide-linked ^{131}I (30) similar to that released in some cases of carcinoma (139) or of thyroiditis (101); the defect at step (*e*) is seen by demonstrating the patient's defective ability to metabolise injected labelled diiodotyrosine (138, 139); and finally, at step (*c*) the flaw is revealed by finding an excess of protein-bound iodine which can be identified as an iodotyrosine (87, 88, 89, 136, 154). In few cases has a corresponding enzyme defect been demonstrated on a slice of thyroid tissue removed at operation (29, 88, 114, 154). In some cases thyroid tissue digests have revealed abnormal proportions of hormonal precursors (29, 88, 114, 154), and subnormal amounts of thyroxine. Hypothyroidism occurs only with severer defects. In these cases, the PB^{127}I or ^{131}I may be high, low, or normal (83, 154), since its proportion of thyroxine is both variable and subnormal; and so this level may not reflect thyroxine output or "thyroid function" On radioiodine testing, these glands show the features of secondary hyperactivity; i e, thyroid hormone administration suppresses the patient's avidity for ^{131}I, albeit slowly at times, and also returns the PB^{127}I to normal. The hypothyroid cases probably

function, and it also permits easier integration with the thyroxine "feedback" control of the other central nervous system influences known to affect thyrotropin secretion. For example, cold and emotional effects might be mediated by a modification in this hypothalamic utilization of thyroid hormones; as an adrenalectomy might change the effects of hypothalamic stimulation from an inhibition to a stimulation of thyroid secretion rate (64); it explains the Goldberg *et al.* evidence (57) that high temperatures or dinitrophenol will inhibit thyroid secretion and prevent pituitary stimulation and goitre development when propyl thiouracil is given (including histological evidence). This action of dinitrophenol on thyroid control is evident in man within 4 hr. on the serum PBI (98).

Thyrotropin (TSH) output and its assay—A sensitive TSH assay, suitable for application to human biological fluids, is still being sought. Two new promising assay methods have been published, and possibly a third (22) Bates & Cornfield (13) have added some refinements to the method of Gilliland & Strudwick (56) by using day-old, fasting chicks, giving them propyl thiouracil as well as thyroxine and shortening the wait before measuring the ^{131}I discharge The Adams & Purves (2) method measures the induced increase in PB^{131}I discharge into the blood of thyroxine-treated guinea pigs McKenzie (89, 90) has adapted this method to mice and so made it the most sensitive one available, an improvement confirmed by Adams (5) It is sensitive to 0 025 mU, per animal, which compares with a sensitivity of 0.1 for the method devised by D'Angelo (28), and of 0 15 mU. for that of Gilliland & Strudwick (56); which means, however, that the probable normal level of 0 16 mU. per ml of serum, is still barely measurable Other less sensitive methods of *in vitro* assay, suitable for larger amounts, have also been published; one based on the weight of thyroid slices (11, 12), and one on the phospholipid turnover response in thyroid slices (47) Thyroid-stimulating hormone has also been measured in urine (19, 75). Freinkel (53) has defined some of the details of action of TSH on phospholipid turnover in thyroid slices Sonenberg (132, 133) has reviewed the chemical and physical properties of TSH

One most interesting finding has emerged from the application of these assays Adams & Purves (1, 3, 4) have demonstrated an abnormal type of TSH found only in the serum of some thyrotoxic patients, particularly those with exophthalmos; and McKenzie (90) has confirmed this This TSH is abnormal in that it produces an exceptionally prolonged discharge of PB^{131}I from the mouse or guinea pig thyroids This abnormal response might be correlated with the finding of an inhibitor to TSH in human urine (142), with the exophthalmos-producing factor of Dobyns & Wilson (31), and with the failure of the thyrotoxic gland's avidity for ^{131}I to be suppressed on administration of thyroid hormone It might also be relevant to the suggestion made by Smelser & Ozanies (130) from guinea pig experi-

TSH actions were evidently not blocked; restoration to normal followed withdrawal. The familiar action of smaller doses of iodide in alleviating thyrotoxicosis has been shown to depend on its blockage of the action of TSH in the thyroid gland (58, 60, 131). Iodide inhibits the increase of the thyrotoxic release rate which normally follows the administration of exogenous TSH to the patient who is receiving blocking doses of antithyroid drugs It also has a similar effect on the hypophysectomised subject who is on a constant TSH dosage and similarly blocked (18).

The Circulating Hormones

Their form—It is now well established (111, 147) that in normal as well as hyperthyroid subjects, most of the protein-bound iodine or circulating thyroid hormone (biologically active thyroid secretion) is thyroxine or T4 (149) with which is probably always admixed about 5 per cent of triiodothyronine or T3 (9, 17, 108) Whether the latter is also secreted from the thyroid or released following peripheral deiodination is not yet settled. More studies are needed of the corresponding compounds in urine (45). Most of the above conclusions have been based on ^{131}I labelling, which has demonstrated few other organic iodine compounds in the blood except in patients with abnormal thyroids. However, Werner & Block (155) have commented that the iodotyrosine pool in the thyroid is large so that these radioactive procedures might miss a small secretion from it, they report finding about half of the normal PB^{127}I to be iodotyrosines.

Thyroxine-binding protein and thyroxine-binding capacity—As is now emerging also for other hormones, it is known that most of the thyroid hormone circulates as bound to a specific protein (TBP), having a high affinity for thyroxine but less affinity for its analogues (111, 119) Additions of thyroxine to serum will saturate this protein, after which the excess moves to a looser binding to the albumin The binding capacity of a serum may be measured from the effect of added thyroxine on the electrophoretic distribution of ^{131}I-thyroxine, from which also can be deduced the level of free thyroxine (6, 106, 118, 119) The purification and the physicochemical properties of TBP have been reported (69, 118, 119) The main TBP is an α-globulin but a second TBP has been found as a prealbumin, whose relation to the main TBP or to the looser albumin-binding is not yet settled (15, 70) A similar thyroxine-binding protein has been demonstrated in the thyroid, which may help to control the gradient between the thyroid and the blood (69), and perhaps a similar mechanism controls the entry of the free thyroxine into the tissues It is the concentration of free thyroxine which probably determines the hormonal drive to the tissues For example, birds which are defective in TBP, respond equivalently to either T4 or T3 (99, 128) The free plasma thyroxine has been estimated at 3 to 9×10^{-11} *M*, and the binding proteins serve to buffer its supply

have a maximal output of TSH, so do not respond to injected TSH (83), and their suppression by thyroid hormone is slow. The importance of the dehalogenase deficiency found in some of these cases is difficult to assess; it could add iodine deficiency to the thyroid's other disabilities, but some of these cases have been found to have normal amounts of iodide circulating (55). Furthermore, high levels of iodotyrosines may be found in the blood without a demonstrable deficiency of this enzyme (154).

Thyroid therapy will often produce remarkable recession in these congenital goitres if they are not nodular (163), and may restore some of the impaired biosynthetic function (94). Zondek (164), though as yet without confirmation, has reported an unusually prolonged response in these cases to infrequent but massive doses of triac (triiodothyroacetic acid). These congenital goitres probably depend on a recessive gene [(66) gives the fullest genetic data], and lesser defects have been shown in their relatives. An interesting special group is that whose deficiency is incomplete enough to be manifested only by a goitre with euthyroidism, of which one special subgroup is associated with congenital deafness (88, 93). This association apparently depends on some genetic connection rather than on a metabolic effect of the thyroid hormone Such cases raise the question, where do these milder cases differ from other non-toxic goitres?

Non-toxic goitre.—The prevalence and geographical distribution of endemic goitre has been recently resurveyed extensively (26, 74). While the incidence in some countries has been largely eliminated by iodine prophylaxis over the last 30 years, it still remains high in many parts of the world. Various studies of iodine metabolism have been made in goitre areas [eg. (115, 135)] which confirmed the presence of iodine deficiency; but similar evidence of deficiency has also been found in the non-goitrous subjects (77, 123). Only rarely have goitrogens been found in the food to explain the goitre—one striking example involving the milk from cows fed on *Brassicae* (25). Studies of the gland hydrolysates from non-toxic goitres have revealed a low di- to monoiodotyrosine ratio, MIT/DIT (110, 150), but while other studies have suggested that this ratio reflects the degree of iodine deficiency (21, 115), even lower ratios have been found in carcinoma (141). Schumacher *et al* (129) have studied the radioiodine incorporation by slices of goitres excised from patients with various diseases and found this function greatly increased in thyrotoxic glands, moderately increased in lymphadenoid goitres, and normal in adenomas

The action of iodide.—An interesting type of drug goitre is that which sometimes follows long periods of administration of large doses of iodide (105, 151, 152) This evidently depends on the local action of iodide in the gland, but iodide inhibits the synthesis of hormone in the rat only for about 24 hr. (161). Paley *et al* (105) showed that while the organic binding was blocked in such a patient, the gland still concentrated iodide so that all of the

mones (TSH, gonadotropin, and growth hormone), and atrophy of all the pituitary target organs as well as decreased growth.

Peripheral Action

The fate of the secreted hormone—The hormones (triiodothyronine, or T3 and thyroxine, or T4) have been shown by both autoradiographic and chromatographic evidence to enter rapidly most of the cells of the body (48, 49, 78). The largest proportion goes to the liver where it is metabolised and produces much of its metabolic effect, as well as also inactivated and excreted into the bile. If large doses are injected, the liver clearance of the hormone rises to 20-fold (96). After bile duct ligation, a greater metabolic stimulus results from the injected hormone, and more of its metabolites are found in the blood, which then includes those which are normally excreted with the bile (46). The human liver clears 200 to 600 ml. of plasma of the hormone per day (95), and the main biliary metabolites are glucuronides. With patients in severe diffuse liver failure, a raised $PB^{127}I$, increased TBP, decreased ^{131}I turnover through the thyroid are found, as is also a slowed disposal from the plasma of ^{131}I-thyroxine (153).

In all tissues, deiodination is probably a part of the hormonal (T3 and T4) degradation process, although further details of this process are needed. It is not certain whether, before exerting its tissue effects T4 must be converted to T3 or modified in other ways, or both. Nor is its mode of action on the tissues known, although it has been shown to modify the activity of a wide range of enzymes, it can interact with metal ions, and it may effect membrane permeability (111)

The action of the thyroid hormones—It is unclear why there is a latent period during which no thyroid hormone produces its action either *in vivo* or *in vitro*. Triac and T3, probably because of their lesser binding by TBP, are perhaps as rapid in action as any known analogue; effects being seen in myxedematous subjects within 4 hr. on the BMR and on the electrocardiogram (51, 67) While the maximum effect of a single dose of triac is seen within 24 hr. and followed by a gradual decline, the daily administration of a maintenance dose causes a steadily rising effect during the first week of its administration (67) Apparently during the restoration of thyroid deficiency, the tissue's responsiveness increases perhaps from induced changes in both tissue enzymes and other endocrine secretions.

Thyroid analogues—Asper & Wilson (10) and Pitt-Rivers & Tata (111) have reviewed the wide range of analogues whose biological activity has recently been studied; those compounds with biological activity vary in potency, and in speed and duration of action, but none has so far shown a qualitatively different biological action 3 3′5′ Triiodothyronine has been found to inhibit thyroxine action (109) Early suggestions that triac could lower blood cholesterol without producing equivalent thyroid effects in other directions, have not been borne out. A similar claim has recently been made for the formic acid derivative (36). Small doses of triac will lower the blood

The concentration of circulating TBP changes little in thyroid disease (6, 119, 148), but it is strikingly raised in pregnancy (33, 34, 121) and lowered in nephrosis (120), probably because of its loss into the urine (52, 54). In pregnancy the TBP is raised from the first or second month and then remains at a constant level until about six weeks after parturition, so that it is probably unrelated to the progressively rising BMR of the later months of pregnancy. Indeed, the deduced free thyroxine level of pregnancy is slightly less than normal; which agrees with the finding that ^{131}I passes more rapidly through the thyroid in pregnancy, but less rapidly thence to the tissues The newborn child shows TBP levels midway between those of the mother and of a normal adult. These TBP changes probably arise from the high estrogen level, since its administration also raises these TBP levels in other females or males (35); while methyl testosterone has an opposite effect on the TBP (43). It may also be noted here that T4 and T3 only cross the placenta minimally (97), and their administration to a mother receiving antithyroid drugs cannot prevent the foetus from developing a goitre (112).

Relation to Other Endocrine Glands

Adrenal.—While most of this inter-relationship still remains obscure (72), two recent studies have clarified one facet—the accelerating effect of thyroid hormone on the liver's metabolism of cortisol with corresponding increases in adrenal secretion rate On infusing cortisol, Peterson (107) found its metabolism more rapid in hyperthyroidism with corresponding changes in the cortisol production rate (^{14}C-cortisol); lesser similar changes were seen in the urinary corticoid output, and the opposite changes were noted in myxedema. After the infusion of cortisol, Brown (23) found the rise of the free 17-hydroxycorticoids and the fall of their conjugates was rapid in thyrotoxicosis and slow in myxedema. On infusion of the corresponding tetrahydro compounds, conjugation was normal in myxedema but rapid in hyperthyroidism. In both conditions, the plasma cortisol is normal, and the 17-ketosteroids low, while the response to ACTH misleadingly varies inversely with thyroid function. This suggests an adrenal-liver-pituitary homeostasis in response to thyroid changes. A clear picture has not yet emerged of the effect of cortisol and ACTH on thyroid function (16). Synergism has been shown between epinephrine and thyroxine secretion in the animal's response to cold (146).

Pituitary—Animal work has clarified the relatively frequent association of hypermetabolism or thyrotoxicosis, or both, with acromegaly (65), and the lower pituitary function of myxedema (27, 41, 42) Growth hormone, TSH via thyroid hormone, and ACTH via cortisol are all calorigenic and inter-related in this effect Growth hormone stimulates thyroid ^{131}I metabolism and also the BMR in hypophysectomised animals; excessive calorigenesis is produced in hypophysectomised animals given both growth hormone and thyroid hormone, which is fatal unless cortisol is also supplied Conversely, thyroidectomy leads to the depletion of the anterior pituitary hor-

giving some indication of the peripheral utilisation of thyroid hormone, may be difficult to interpret because of the influences of the TBP level, the concentration of free thyroxine, liver function, etc. This procedure indicated that peripheral deiodination of T4 was not a source of the serum T3 (79). Thyrotoxic plasma disposed of its labelled hormones more rapidly than did normal plasma enriched to the same concentration of hormone (62). Sterling found that both the slower turnover of myxedema and the faster turnover of thyrotoxicosis returned to normal on the restoration of thyroid function (143); while Ingbar found this rate still raised in treated thyrotoxic subjects and also in their euthyroid relatives (68, 71) This hormonal disposal rate has also been reported high in otherwise euthyroid subjects with paroxysmal cardiac arrhythmias (76).

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cholesterol of myxedematous subjects without easily discernible other effects, but so will T3 and T4 if given in equivalent dosage. However, very small doses of triac have precipitated angina in susceptible subjects even within 2 hr. of the first dose (67, 85, 100), possibly more readily than T4. These rapidly-acting compounds nevertheless have a place in the treatment of myxedema coma. This condition has often been fatal, possibly because of the high dosage of thyroid hormone given (7, 37); moderate dosage has been attended with temporary success before a final myocardial infarct (145).

Radioiodine Tests

Among the main tests of thyroid function, radioiodine now has its established place (50) and appropriate procedures can measure the various steps in the iodine cycle through the body (116) The normal iodine uptake has been shown to vary with age as does the BMR (84), although clinical standards may not need such a fine correction; an adaptation by the thyroid to decreased tissue utilization has been shown to be the basis of this change (157) Details are available of the timing of the different human responses to injected TSH and of the effect of dose (38).

To distinguish thyrotoxicosis from other goitres which are avid for iodine, two interestingly different iodide response tests have been established, an iodide repletion test (24) and an iodide inhibition test (44). In the former, ^{131}I retesting at four weeks after two weeks of daily 10 mg doses of KI, reveals thyrotoxic glands still avid for ^{131}I, but the others show a normal response In the second test, a smaller dose of iodide (1 to 2 mg) is added [illegible] hibition.

The thyroid hormone (usually T3) suppression test continues to be a useful method for making the same distinction as these two iodide tests Johnson (73) has shown that the normal response on this test includes suppression of both discharge and uptake; while the thyrotoxic fails even after very high doses, so implying a qualitatively disordered regulation Other associated changes seen during the test have also been studied (117).

The measuring *in vitro* of the entry of T3 into the patient's red blood cells is shown on a large series to separate without overlap high, normal, and low thyroid function (63) However, abnormal results are also found with altered TBP (in pregnancy, after estrogen, in nephrosis, and in liver disease), as well as for unexplained reasons in pulmonary insufficiency, and after anticoagulants; a normal result was found with iodide myxedema Analogously, red blood cells labelled with ^{32}P lose this label more rapidly in thyrotoxicosis, and less rapidly than normal in myxedematous subjects (39, 40)

Measurements of the rate of disposal of injected ^{131}I–T4 or –T3, while

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HORMONAL CONTROL OF PIGMENTATION[1,2]

By Aaron B. Lerner, M D

Section of Dermatology, Department of Medicine, Yale University School of Medicine, New Haven, Connecticut

At the turn of the century two French investigators, Bertrand & Bourquelot (1, 2), showed that a plant enzyme which they named tyrosinase could catalyze the oxidation of the amino acid tyrosine to a dark product. Subsequently tyrosinase was found in melanomas of man and animals. In the 1920's, the extensive researches of Bloch in Switzerland and Raper in England established the fact that tyrosinase played an important role in melanin formation in mammals (3) As a result of these studies, physicians, particularly in Europe, tried to explain abnormalities in pigmentation through alterations in tyrosinase function. During this period biologists, principally in the United States, carried out experiments that were to lay the foundation for our understanding of hormonal control of pigmentation The biologists showed that in animals such as tadpoles and frogs removal of the pituitary gland resulted in marked lightening of skin color, whereas injection of pituitary extracts produced darkening of skin color Also, it was shown that pineal extracts lightened the skin color of tadpoles and frogs. Unfortunately, clinicians of that period paid little attention to biological studies; few or no attempts were made to explain pigmentary abnormalities on the basis of alterations in function of the pituitary gland and the nervous system It was too difficult to conceive of a relationship between the dramatic changes caused by hormones on the skin of tadpoles and frogs and the much less dramatic changes that occur in man

In the last f [illegible]

in hormonal control of melanin pigmentation We can explain many normal and abnormal pigmentary states in man We may have the opportunity to change man's coloring at will.

In this report we will discuss the color changes in adrenocortical insufficiency and vitiligo as well as some interesting miscellaneous disorders. Hyperpigmentation resulting from androgenic and estrogenic stimuli will not be reviewed.

BASIC CONSIDERATIONS

At least two melanocyte stimulating hormones are produced by the pituitary glands of animals that have been studied (4). Thus far, there is

[1] The survey of the literature pertaining to this review was concluded in September, 1959

[2] The following abbreviation will be used: MSH (Melanocyte Stimulating Hormone).

TABLE I

AMINO ACID SEQUENCES OF MELANOCYTE STIMULATING HORMONES (α- AND β-MSH) AND CORTICOTROPIN (ACTH)

The complete sequences of human, bovine, and ovine ACTH have not been worked out yet

α-MSH (porcine, bovine) 13 amino acids	CH_3COSer Tyr Ser Met Glu His Phe Arg Try Gly Lys Pro ValNH_2
β-MSH (porcine, ovine) 18 amino acids	Asp Glu Gly Pro Tyr Lys Met Glu His Phe Arg Try Gly Ser Pro Pro Lys Asp
β-MSH (bovine, ovine) 18 amino acids	Asp Ser Gly Pro Tyr Lys Met Glu His Phe Arg Try Gly Ser Pro Pro Lys Asp
β-MSH (human) 22 amino acids	Ala Glu Lys Lys Asp Glu Gly Pro Tyr Arg Met Glu His Phe Arg Try Gly Ser Pro Pro Lys Asp
ACTH (porcine) 39 amino acids	Ser Tyr Ser Met Glu His Phe Arg Try Gly Lys Pro Val Gly Lys Lys Phe Glu Leu Pro Phe Ala Glu Ala Leu Glu Asp Glu Ala Gly Asp Pro Tyr Val Lys Val Pro Arg Arg
ACTH (bovine) 39 amino acids	Ser Tyr Ser Met Glu His Phe Arg Try Gly Lys Pro Val Gly Lys Lys Phe Glu Leu Pro Phe Ala Glu Ala Ser Asp Glu Ala Glu Gly Asp Pro Tyr Val Lys Val Pro Arg Arg
ACTH (ovine) 39 amino acids	Ser Tyr Ser Met Glu His Phe Arg Try Gly Lys Pro Val Gly Lys Lys Phe Glu Leu Pro Phe Ala Glu Ser Ala Glu Asp Asp Glu Gly Ala Pro Tyr Val Lys Val Pro Arg Arg
ACTH (human) 39 amino acids	Ser Tyr Ser Met Glu His Phe Arg Try Gly Lys Pro Val Gly Lys Lys Phe Glu Leu Pro Phe Ala Glu Ala Ser Glu Asp Glu Ala Gly Asp Pro Tyr Val Lys Val Pro Arg Arg

only one α-MSH, but there are three β-MSH's (Table I), (4, 5). All the MSH's have been isolated through the use of some type of frog skin assay. Bovine and porcine α-MSH is the most potent darkening agent known. It is a linear peptide composed of 13 amino acids. These 13 amino acids are the same as the first 13 amino acids of adrenal corticotropic hormone (ACTH) obtained from human, bovine, porcine, and ovine pituitary glands; *and they are in the same sequence as those in* ACTH (6a, b, c, d). However, there are two subtle differences between the 13 amino acid chain of [illegible]

a molar basis ACTH has only 1/30 the darkening activity of α-MSH. However, when the N-terminal serine of ACTH is acetylated, it has one-fifth the darkening activity of α-MSH. A sixfold increase in darkening activity results merely from putting an aceytl group on the N-terminal serine of ACTH.

Recently, Hofmann *et al.* synthesized α-MSH with the glutamic acid in the form of glutamine and a formyl group on lysine (7). Synthetic α-MSH is the largest peptide made thus far. It has about the same activity as the natural hormone. Guttmann & Boissonnas have synthesized α-MSH without any blocking groups (8). The biologic activity of this α-MSH has not been compared directly with the natural hormone.

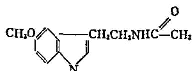
Beta-MSH from hogs has about one-third the activity of α-MSH. As yet, human and bovine β-MSH have not been compared accurately with α-MSH or porcine β-MSH. Human β-MSH is somewhat larger than the other β-MSH's. Another MSH is present in human pituitary gland, but whether or not it is of the α-type remains to be seen.

Injection of a crude mixture of porcine α- and β-MSH produced Addison-like hyperpigmentation in man (9) and darkening of melanomas in hamsters (10). Pure α- and β-MSH have not been given yet to human subjects.

There are darkening hormones other than α- β-MSH, and in special instances they may be very important. Both androgens and estrogens under the appropriate conditions can darken human skin *in vivo*. These substances have no effect on frog skin. However, progesterone is a weak but definite *darkening agent of frog skin. Recently a systematic search for darkening* agents in bovine organs was carried out using a frog skin bioassay (11). Potent darkening factors were present in the thyroid gland, small intestine, pancreas, and lungs.

While many investigators have been interested in darkening agents, only a few have studied lightening factors. Melatonin (N-acetyl-5-methoxytryptamine), present in the pineal gland and in peripheral nerves, shows unusual potency in lightening frog skin (12 to 14). For some time epinephrine and norepinephrine were considered to be the most active lightening agents known. However, melatonin is about 100,000 times more active. Acetylcholine has the same activity as epinephrine and norepinephrine

Less active are serotonin and triiodothyronine It seems significant that the most potent lightening agent, melatonin, and the most potent darkening agent, α-MSH, are both N-acetyl compounds Crude but active melatonin preparations lighten the color of hamster melanomas and frog skin (10). They decrease melanin formation in frog melanocytes. As yet, melatonin has not been given to man. However, this neurohormone may be the factor in peripheral nerve endings that causes vitiligo (15). A decrease in melatonin in the nerves may be associated with an increase in pigmentation.



MELATONIN

Before describing the clinical aspects of pigmentary disorders two seemingly paradoxical situations will be mentioned, and suggestions will be offered to explain them. In some disorders both hyperpigmentation and hypopigmentation are present This is particularly true of Addison's disease in which both severe hyperpigmentation and extensive vitiligo may occur simultaneously. The other unusual finding is that patients with total alopecia and complete vitiligo excrete excessive amounts of MSH in the urine (16). In spite of an output of large quantities of MSH, these patients' have hypopigmentation, not hyperpigmentation. A possible explanation of the first case is that in some individuals with hypofunction of the adrenal medulla there may be not only a compensatory increased output of norepinephrine by the peripheral nerves, but also of an agent that could cause hypopigmentation. A possible explanation of the second case is that in complete vitiligo the excessive release into the systemic circulation of a neurogenic pigment cell lightening agent could stimulate MSH production by the pituitary gland. The increased MSH would not be sufficient to counteract the skin lightening effect of the agent. This case is not unlike that in which norepinephrine given intravenously to man results in an increased output of MSH in the urine (17). Yet, norepinephrine itself is a good lightening agent *In vivo* systems are more complicated than *in vitro* ones. The net effect of injecting a lightening or darkening agent depends on that agent's ability to alter melanocytes directly versus its ability to induce changes in other organs which, in turn, have an opposite action on melanocytes.

CLINICAL CONSIDERATIONS

MELANOCYTE STIMULATING HORMONE DARKENING

Hyperpigmentation resulting from an excess output of MSH occurs in three conditions: (*a*) adrenocortical insufficiency; (*b*) Cushing's syndrome in which hyperpigmentation becomes evident only after bilateral

adrenalectomy and subsequent growth of a pituitary tumor; and (*c*) pituitary tumor that releases MSH but not enough ACTH to produce Cushing's syndrome.

Adrenocortical insufficiency—All types of adrenocortical insufficiency whether caused by tuberculosis (Addison's disease), idiopathic atrophy, adrenalectomy, etc, are associated with hyperpigmentation as long as pituitary function is not impaired and adequate supportive therapy with a hydrocortisone analogue is not maintained. This hyperpigmentation is usually characteristic. Darkening occurs in the exposed areas, body folds, mucous membranes, and sites of recent trauma (Fig. 1). Hydrocortisone and perhaps other steroids from the adrenal cortex inhibit the output of MSH from the intermediate lobe of the pituitary gland as well as ACTH from the anterior lobe. Usually 30 to 40 mg. of hydrocortisone orally are required in bilaterally adrenalectomized patients in order to suppress the

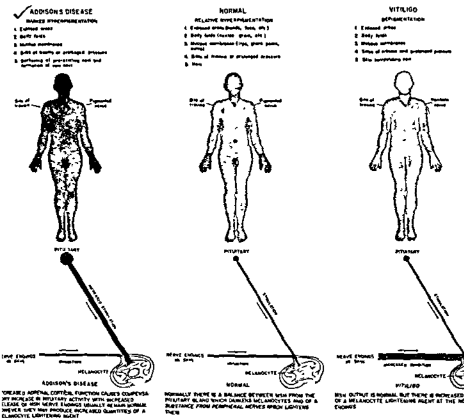


FIG. 1. FROM LERNER (15) WITH PERMISSON OF THE WILLIAMS & WILKINS CO

output of these peptides from the pituitary gland When adrenocortical function is impaired and formation of hydrocortisone decreases, there is a subsequent rise in MSH production which is followed by darkening of the skin (9). As mentioned earlier, patients with adrenocortical insufficiency and hyperpigmentation are predisposed to vitiligo (15). Indeed, if a patient shows both marked hyperpigmentation and vitiligo, he very likely has adrenocortical insufficiency.

Cushing's syndrome treated by adrenalectomy.—In most instances, Cushing's syndrome results from hyperplasia of one or both adrenal glands. Even when only a single adrenal gland seems to be involved, bilateral adrenalectomy in one or two stages usually is the treatment of choice because eventually both glands become hyperplastic or undergo neoplastic changes. Most patients do well on steroid therapy following surgery, and no hyperpigmentation occurs. However, physicians wrote us about four patients (18), and two cases have been reported (19, 20), all of whom had an initially good response to adrenalectomy but developed extreme hyperpigmentation in spite of steroid therapy. Nelson (21) has collected a total of 13 such patients including the preceding six. Concerning their patients, the attending physicians commonly remarked, "This individual is the darkest Addison's patient I have seen." Subsequently these patients were found to have pituitary neoplasms. The latter probably were MSH-producing tumors; however, urine of only one patient was assayed for MSH. The latter was markedly elevated as was the ACTH output (19). While some clinicians were of the opinion that the pituitary tumor was caused by bilateral adrenalectomy, it seems more reasonable that a small pituitary tumor caused the adrenal cortical hyperplasia; and with time this tumor enlarged.

Primary MSH-producing pituitary tumors. We examined one patient and received secondhand knowledge of another who had pituitary tumors without laboratory or clinical evidence of Cushing's syndrome but with pronounced darkening of the skin Urine MSH levels were elevated In our patient, and perhaps in the second as well, the tumor produced an excessive amount of MSH but not ACTH Thus, there was hyperpigmentation without adrenocortical stimulation

Non-Melanocyte Stimulating Hormone Darkening

Hyperpigmentation similar to that produced by increased amounts of MSH sometimes is seen in patients with hyperthyroidism, biliary cirrhosis, sprue, and other diseases. *Does MSH play an important role in producing hyperpigmentation in these conditions?* The answer is not known at the present time. Recently, various bovine organs were screened for darkening agents While pituitary extracts—primarily because of MSH—were the most potent, darkening factors also were found in the thyroid gland, small intestine, pancreas, and lung The thyroid factor which was very active

was not MSH It is not known yet whether darkening substances in the other organs are related to MSH. It is possible that in some patients with hyperthyroidism an increase in melanin pigmentation results directly from a thyroid darkening factor, from MSH or from a decrease in melatonin.

Vitiligo

Vitiligo is a common and important disorder in which pigmentation is decreased in some parts or all of the skin (15) Hypopigmentation occurs in the exposed areas, body folds, mucous membranes, and sites of recent trauma (Fig. 1). Thus, in vitiligo the usual areas of depigmentation are the same as the usual sites of abnormal hyperpigmentation seen in patients with increased MSH output. At times there is complete loss of pigment from the skin and even from the hair. The eyes retain their pigment. Most patients with vitiligo are otherwise in good health. However, patients with hyperthyrodism, pernicious anemia, adrenocortical insufficiency, sympathetic ophthalmia, and neoplasms are predisposed to vitiligo An interesting syndrome consists of total alopecia and complete vitiligo. Frequently, in this situation hypopigmentation does not appear in the usual patchy distribution of ordinary vitiligo but, instead, suddenly involves the entire body. Patients with this combination excrete elevated quantities of MSH in the urine, yet have hypopigmentation and not hyperpigmentation It is possible that in these individuals the presence of abnormally large amounts of a skin-lightening substance is more significant than the darkening effect of MSH. Vitiligo may result from excessive release of such a factor by peripheral nerves in the region of the melanocytes Whether or not this long sought for lightening factor is melatonin is not yet known.

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BIOCHEMICAL CHANGES IN THE DERMATITIDES[1]

By Arthur C. Curtis, M D. and Walter D. Block, Ph.D.
Department of Dermatology, University of Michigan Medical School, Ann Arbor, Michigan

This review deals with studies of basic biochemical processes in normal human skin and in various dermatoses. No attempt has been made to include studies concerned primarily with clinical description or therapeutic treatment of skin diseases. Publications appearing from January, 1957, through December, 1958, are the main basis for this review.

Proteins

Within the last few years the fractionation and characterization of the proteins of human skin have been studied extensively. Matoltsy and his group (1, 2) isolated two proteins from neutral phosphate buffer extracts of human plantar skin (callus). Each protein was homogeneous when subjected to electrophoresis but heterogeneous when subjected to ultracentrifugation. Whether one of the proteins was a precursor of epidermal keratin or whether it represented a natural degradation product of decomposing cornified cells, was left for further studies. Unlike Rudall (3), who found that 6 *M* urea extracted from bovine epidermis a specific fibrous protein ("epidermin"), Matoltsy & Herbst (4) using human epidermis were unable to obtain with urea extraction a fibrous protein which could not be obtained by the use of other protein extractants. Roe (5) was able to extract a fibrous protein from human epidermis with 75 per cent lithium bromide. This fibrous material [illegible]

the Keratin, Myosin, Elastin, Fibrinogen (KMEF) group.

Evidence is accumulating which indicates that skin contains proteins having some characteristics similar to the proteins of blood. Zimmer & Woringer (7) subjected thin slices of whole human skin, imbedded in starch gel, to electrophoresis and found that they contained an albumin and a γ-globulin whose migration pattern resembled those present in serum. Skin also contained an α- and β-globulin different from those in serum. Hemoglobin was detected but was thought to be a contaminant because the intensity of the stained protein band decreased as the layer of skin used was *further removed from the large blood vessels On the other hand,* the stained protein band corresponding to β-globulin varied inversely in intensity to that of the hemoglobin. The authors concluded from this that β-globulin was part of the tissue and not present because of an interchange with serum. The work of Humphrey, Neuberger & Perkins (8) with rabbit skin, and of

[1] The survey of literature pertaining to this review was concluded in March, 1959.

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21. Nelson, D. H (Personal communication to the author)

of radioactive phosphorus incorporated from sodium phosphate-^{32}P into RNA of whole skin of young rats was three times that incorporated into DNA.

Metabolic studies and studies of the blood constituents of patients suffering from various dermatoses have been done by several laboratories. Block *et al.* (28) reported neither undue retention nor loss of nitrogen by psoriatic patients given a normal level of protein intake. Sulfur metabolism appeared to be normal except for the amount of conjugated sulfur excreted Price and his co-workers (29, 30) found that patients with scleroderma (acrosclerosis)

abnormally to test loads (2 gm.) of the amino acid by excreting markedly large amounts of all the metabolites except xanthurenic acid which remained normal Excretion of the metabolites was returned to nearly normal in the patients with scleroderma by the administration of a chelating agent (disodiumethylenediaminetetraacetic acid) which, the authors postulated, made magnesium more available in order to activate the tissue kynureninase, an enzyme involved in the early steps of tryptophan metabolism. Simultaneous administration of pyridoxine, a cofactor for the enzyme kynureninase, was also beneficial. Lea, Curtis & Bernstein (31) were unable to find abnormal levels of serum uric acid in psoriatic patients Lipoprotein levels in sera from psoriatic patients were within normal range (32, 33, 34).

Lipids

With the development of gas-liquid chromatography, investigations of surface and epidermal lipids in human skin have been greatly stimulated. Using this technique, James & Wheatley (35) reported two series of branched chain fatty acids in surface lipids collected from the human forearm. The first series was thought to be highly branched and to consist of odd numbers of carbon atoms; the second series possessed only a methyl branch and occurred with both odd and even numbers of carbon atoms. Wheatley and his group (36) later showed that epidermal lipids ("Malpighian layer lipids" extracted from epidermis which had the stratum corneum removed), unlike surface lipids, did not contain significant amounts of odd-numbered or branched chain fatty acids, but contained predominately unsaturated C_{18} acids.

Herrmann *et al.* (37) reaffirmed the direct relationship between the extent of spreading and the content of free fatty acids of surface lipids obtained from normal human subjects. The same relationship was not observed with surface lipids obtained from uninvolved skin of psoriatic patients

Boughton *et al.* (38), confirming the work of others, reported wide variations in the content of squalene in sebum, both from day to day and between different individuals These workers postulated that this variation could be due to excretion through the skin of squalene in excess of that

Cooper & Johnson (9) with bovine hide has indicated interchange between the proteins of the blood and those of the skin Approximately 60 per cent of the skin albumin of rabbits was replaced daily by serum albumin (8).

Roe (10) isolated three classes of proteins (fibrous, glyco- and nucleo-) from a borate extract of psoriatic scales The fibrous protein was thought [illegible]

lower than in psoriatic scales. Non-psoriatic exfoliative scales contained only the glycoprotein. None of the three types of proteins was found in callus Flesch & Esoda (11) obtained a protein material from water extracts of psoriatic scales different from that obtained by similar treatment of callus or scales from exfoliative dermatoses

The amino acid content of whole human skin, dermis, epidermis, callus, and scales from several dermatoses has been determined by various laboratories (12 to 16) Values found for the different layers of skin vary widely from laboratory to laboratory and thus are difficult to compare. The use of these data to evaluate any change in amino acid composition because of the keratinization process is of questionable accuracy. Hähnel (17) reported that qualitatively there was no difference between the N-terminal amino acids in the proteins of callus and psoriatic scale.

Psoriatic scales and postultraviolet irradiation scales from a normal individual had similar distribution of free amino acids according to Cornish, Block & Lea (18); sulfur-containing amino acids were not found. Flesch & Esoda (19) found no difference between the free amino acid composition of extracts of psoriatic scales and callus, but the free amino nitrogen content of the scales was lower (20) Woringer & Zimmer (21) reported that keloid tissue contained more free amino acids and less peptides than normal human skin.

The presence in skin of nitrogenous compounds related to amino acids and protein is under continuing study. The histamine content of normal human skin from different areas of the body was found by Johnson (22) to have a five- and sixfold variation. Acid hydrolysis of human abdominal skin caused a threefold increase in the histamine content. Davis *et al.* (23) and Sjoerdsma *et al.* (24) reported an elevated amount of histamine but a normal amount of 5-hydroxytryptamine (serotonin) in involved skin from one patient with urticaria pigmentosa.

Rodesch & Mandel (25, 26) reported the quantitative distribution of [illegible] of normal white rats, Rats deprived [illegible] es (^{32}P uptake [illegible] They degraded DNA at a reduced rate parallel to its synthesis, with the result that the DNA content in the tissues remained normal On the other hand, RNA was degraded faster than it was synthesized and thus the RNA content was lowered by protein deprivation. Bernstein & Foster (27) found that amounts

Lever & Klein (47) reported that patients with idiopathic hyperlipemia showed delayed elimination of intravenously administered fat emulsions when compared with primary hypercholesterolemic patients and normal individuals. Holt (48) found no evidence of delay in fat metabolism when ^{131}I-labeled olive oil was fed to a seven-year-old boy with idiopathic hyperlipemia

Lever & Waddell (49) reported earlier that the intravenous administration of a cottonseed oil preparation led to a temporary drop in serum lipids in patients with idiopathic hyperlipemia. This work was confirmed by Everett, Block & Curtis (50). Oral administration of large amounts of unsaturated fatty acids was reported to have the same effect by Everett *et al.* (51). Further work in this area and on the effect of heparin in serum lipid clearing in normal subjects and in patients with idiopathic hyperlipemia has continued (47, 50, 52 to 54) Studies by Lever and his group showed that inhibitors of lipemia clearing activity are present in the serum of patients with hyperlipemia (54) and in extracts of human and rabbit spleen, kidney, and liver (55) Engelberg (56) reported that lipolytic activity of human postheparin and endogenous plasma was inactivated during the process of [illegible]

(57) reported that oral administration of gastric mucin, which contains an acid mucopolysaccharide composed essentially of D-glucuronic acid and D-glucosamine had a plasma clearing action. The mechanism was not known.

An excellent review concerning heparin and blood lipids has been written by Levy (58)

Carbohydrate Metabolism

The failure to demonstrate the *in vitro* oxidation of both citrate and ketoglutarate, and the inability to show the presence of isocitric dehydrogenase in skin slices and homogenates by Barron *et al.* (59) in 1948 have led to some question as to whether the Krebs cycle, as such, operates in the aerobic utilization of carbohydrate by this tissue Recently Cruickshank, Hershey & Lewis (60), using a newly developed micro-method, reported that isocitric dehydrogenase was present in significant amounts in epidermis of human skin. With the demonstration of the presence of isocitric dehydrogenase in skin, it is unnecessary to postulate alternate pathways for citrate utilization. This, together with previous reports of fumarase, lactic dehydrogenase, malic dehydrogenase (61), succinic dehydrogenase (62), and coenzyme A (63) occurring in human skin, is additional evidence that the Krebs cycle can operate in this tissue (60).

Elevated succinic dehydrogenase activity has been noted in skin from patients with Duhring's disease and psoriasis (62, 64); in pemphigus the activity tended to be reduced (62). Zina & Bonu (63) reported abnormally high amounts of coenzyme A in the epidermis of lichenified skin (neurodermatitis); psoriasis scales and parakeratotic scales contained amounts lower than found in normal epidermis.

needed for cholesterol synthesis. Reports that squalene inactivated sulfhydryl groups, inhibited certain enzymes, and had bactericidal and fungicidal action as was postulated by earlier workers, could not be confirmed.

Windhorst & Foster (39) reported low concentrations of free and total sterols in lipids collected from the scalps of balding men In contrast, lipids from the scalps of women were high in sterols, and those from non-balding men were intermediate.

In normal human adult subjects, Reinertson & Wheatley (36) found that the content of all sterol fractions (free and total cholesterol, 7-dehydrocholesterol, and other Liebermann-Burchardt "fast-acting" sterols) was higher in the "Malpighian layer lipids" than in those from stratum corneum or total epidermis. Extremely high amounts of total sterols and 7-dehydrocholesterol were found in epidermis from a two-week-old infant as compared to that of normal adults. In uninvolved areas of epidermis taken from psoriatic patients, the content of total lipid and total sterol was normal; the "fast-acting" sterol content was low. The cholesterol ester to total cholesterol ratio was normal. The uninvolved epidermis also contained an unidentified, lipid-soluble substance which "traveled" together with free sterols on a chromatographic column, and had an ultraviolet absorption spectrum between 260 and 290 μ. This substance was not found in normal skin. Cornish, Block & Lea (18) reported values for total lipids, cholesterol, and esterified cholesterol in psoriatic scales similar to normal callus.

Total phospholipid content in normal "Malpighian layer lipids" was ninefold that found in the stratum corneum but only slightly higher than that of total epidermis (36) In psoriatic scales, phospholipid amounts were greater than in normal callus (18). Suzuki (40) showed *in vitro* that whole skin slices from axillae of patients with osmidrosis incorporated labeled phosphate into phospholipids. The presence of phosphatides in sebum has been reported (41).

Investigations of the role of fatty acids in dermatoses have continued. Hansen and co-workers (42) again showed that young infants develop abnormal skin manifestations when fed diets low in essential fatty acids. The addition of saturated fatty acids to the diet caused no improvement. On the other hand, the addition of linoleic acid as trilinolein (2 per cent of daily caloric intake) restored the skin to normal appearance in one to two weeks Sinclair (43) reported serum hexaene values of zero and low amounts of serum dienes and tetraenes in cases of pityriasis rubra pilaris.

The study of primary hypercholesterolemia and idiopathic hyperlipemia continues. Guravich (44) and Hood & Angervall (45) reported clinical and biochemical findings in a large number of patients with primary hypercholesterolemia. That either oversynthesis or decreased utilization of cholesterol may cause hypercholesterolemia, at least in part, was indicated by the findings of Leonhardi (46). Orally administered ^{14}C-acetate labeled in the carboxyl group was not only incorporated into serum cholesterol by a primary hypercholesterolemic patient at a more rapid rate than normal, but the radioactivity disappeared from the serum cholesterol at a slower rate

Enzymes

The existence of significant amounts of protease in human epidermis was first shown by Wells & Babcock in 1953 (81). Recently, a number of publications concerning the identification of specific proteases in skin have appeared. Paschoud, Schmidli & Keller (82, 83) reported that extracts of normal human skin and of uninvolved skin from psoriatic patients contained similar amounts of di- and tri-peptidases Extracts of involved psoriatic

inhibitor. Elevated peptidase activity was also found in extracts of skin of patients with subacute to chronic inflammations. In addition, extracts of human skin contain endopeptidases (84) which show increased activity in the presence of small amounts of heparin and elheparin (85) according to Stuttgen, Hofmann & Simmich In a series of publications, Martin, Axelrod and their co-workers (86 to 90) described the separation, purification, and characterization of four endopeptidases from acetone powder of whole skin of rats Of the four enzymes, the only one which occurred in extracts of fresh human abdominal skin was the enzyme which split acetylated aromatic amino acid esters (91)

The relationship of proteolysis to blister formation was studied by Stoughton & Novak (92) who were able to cause separation of epidermal cells and blister formation in human skin slices by exposing them to 55°C. for 1 to 2 min followed by incubating at 37°C. Presumably an enzymatic factor which digested the intercellular bridges was released In support of this, di- and tri-peptidases have been shown to exist in fluid from blisters caused by pemphigus, burns, and cantharidin (93, 94) Further, Dougherty *et al* (95) by injection of the endopeptidase, trypsin, produced hemorrhagic vesicles in skin of normal subjects. Because less trypsin was needed to form vesicles in pemphigus patients the authors postulated that more proteases than normal must exist in the skin of these patients

A relationship between the severity of the dermatitis and the protease level of the blood has been reported by Dougherty *et al* (95) who found that pemphigus patients had elevated plasma fibrinolysin Braun-Falco & Salfeld (96) noted abnormally high values for leucine amino-peptidase activity in serum from patients with pemphigus, eczema, and neurodermatitis diffusa, whereas serum of patients with psoriasis had normal leucine amino-peptidase activity.

The mechanism of the "itch sensation" in man involves release of skin intracellular proteases which act directly on nerve endings according to Shelley & Arthur (97) The effects of several endopeptidases on itch response in normal subjects and in patients with dermatoses have been investigated (97 to 100) Monash & Woessner (101), in contradiction to the proteolytic enzyme theory, reported that spicules of *Mucuna pruriens* which had no proteolytic activity because of heat inactivation caused sensations of

Glucose utilization by skin continues to be studied. Cruickshank, Trotter & Cooper (65) reported that total glucose utilization by whole guinea pig skin was 4.44 µg./mg./hr. with 2.33 µg./mg./hr. of lactic acid produced over a 5-hr. period. These values are considerably lower than reported previously by Cruickshank & Trotter (66).

Bernstein & Sweet (67) confirmed and extended the findings of earlier workers in reporting the existence of most of the enzymes of the glycolytic system in "crude" extracts of whole skin of young white rats.

The use of glycogen by sweat glands has been investigated by Dobson *et al.* (68), using histochemical techniques. They reported diminution of sweat gland glycogen in human subjects confined in a sweat box for 1 hr.; after 6 hrs. of sweating, the glands were essentially glycogen free. Non-specific esterase activity (substrate, Tween-60) showed a definite increase as the glycogen became exhausted.

Further work (69) has been done showing the Q_{O_2} of human epidermis to be higher than dermis and smooth muscle, but lower than tonsil, gastric mucosa, and cerebral cortex. In a limited number of samples, the Q_{O_2} values of skin obtained from patients with psoriasis, pemphigus, and neurodermatitis were within the range of normal.

Mucopolysaccharides

By use of isotopic labeled compounds, the metabolism of hyaluronic acid and the sulfated mucopolysaccharides in rat and rabbit skin has been studied by Dorfman and his group (70). Using ^{14}C uniformly labeled glucose or ^{14}C carboxyl-labeled acetate and ^{35}S-labeled Na_2SO_4, turnover rates were determined for hyaluronic acid (2.4 to 4.5 days) and chondroitin sulfate (7.6 to 10.7 days) (71 to 73). Turnover rates and uptake of the labeled compounds in skin from alloxan diabetic rats were both lower than found in normal animals (74). Insulin treatment restored the values toward normal. According to Roden & Dorfman (75), who used ^{14}C-glucose labeled in the sixth position, the L-iduronic acid of chondroitin sulfuric acid-B of rat skin was derived from glucose without splitting of the carbon chain.

Denko & Stoughton (76) reported that skin of patients with progressive scleroderma bound intravenously administered radioactive sulfur from $Na_2{}^{35}SO_4$ at an abnormal rate. Instead of normal fixation occurring in 24 to 48 hr., and then a regular decrease, there was a progressive increase in fixation by the skin over a period of about 30 days. Skin from a patient with localized scleroderma showed a much lower rate of ^{35}S fixation than for normal skin. Beierwaltes & Bollett (77) reported that the acid mucopolysaccharide content of involved whole skin from patients with "localized" pretibial myxedema was five to six times greater than that of uninvolved skin from the same individuals. Moreover, a significantly higher amount of acid mucopolysaccharide was found in the uninvolved skin of the patients as compared to whole skin of normal persons. Steiner (78 to 80), using histochemical techniques, has reported a detailed study of the mucopolysaccharide content of cutaneous connective tissue in various dermatoses.

Priestley & Foster (114) determined the ascorbic acid content of normal human epidermis.

Investigations of the content of minerals in human skin continue to be active Stitch (115) noted that aluminum and copper occurred in relatively high amounts, and manganese and rubidium in low amounts in human skin as compared with other soft tissues of the body. Sowden (116) has published values for the barium and strontium content of human skin. Fregert (117), in a reinvestigation of the silicon content of human skin, found that almost 40 per cent of the epidermal silicon occurred in the cornified layer. Felsher (118) showed that skin chloride content in dermatitis herpetiformis and in pemphigus was normal. Reinberg *et al* (119) reported that the potassium content of involved skin of psoriatic patients was higher than uninvolved skin or skin from normal subjects; it seemed to be related to the rate of cell proliferation.

The permeability of human skin to calcium ions and to sulfur has been measured by the use of radioactive isotopes of these substances. Stuttgen & Betzler (120) showed that the permeability of human skin to ^{45}Ca ions was greater in the direction of dermis to epidermis than in the opposite direction. Scott (121) found that ^{35}S elemental sulfur applied topically penetrated normal skin and the uninvolved skin of patients with various dermatoses at a similar rate, reaching the dermis and showing no localization in any specific layer of the epidermis More rapid penetration was noted in the involved skin of patients with seborrheic dermatitis, psoriasis, and eczema The radioactive sulfur tended to become localized in the stratum granulosum and in the basal layer, respectively, in the first two dermatoses There was no localization in eczema

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itching in normal subjects, and that spicules which had proteolytic activity but had been exposed to moisture no longer caused itching.

Protease activity of plasma does not seem to be related to itching according to Cormia *et al.* (102), who found that patients with severe pruritis had slightly elevated plasma protease activity. A comparison between proteolytic activity in blood before and after trypsin injection in patients with pruritis and in normal subjects showed no significant variation.

An elaborate and comprehensive histological study of alkaline phosphatase distribution in normal human skin and in a large series of dermatoses has been published by Kopf (103). In general, the enzyme was found in those areas of tissue undergoing rapid proliferation. Kopf & Orentreich (104) found that alkaline phosphatase activity was below normal in the early stages of alopecia areata but was increased above normal in the late stages of this disease and in alopecia totalis. They believed, however, that loss or lowering of the enzyme activity was not the cause of the alopecia but was only one of the biochemical alterations taking place.

The enzyme, arginase, occurs in human skin, mainly in the epidermis (105). Recently, Rothberg and co-workers (106, 107), studying the arginase activity of normal skin from different areas of the body found that plantar stratum corneum was five to six times more active than trunk stratum corneum. Elevated arginase activity was observed in scales from various dermatoses (psoriasis, exfoliative erythroderma, nummular eczema, ichthyosiform erythroderma, and ichthyosis) possibly because of the absence of an inhibitor such as exists in normal epidermis.

Reviews discussing enzymes and their clinical implications in skin (108) and their roles in allergy (109) have recently been published.

Vitamins and Minerals

The biochemical role of vitamins in human skin has received little attention during the last several years Greenberg, Cornbleet & Demovsky (110) found moderate amounts of vitamin A in sebaceous glands, sebum of hair follicles, and on the skin surface after intradermal injection of a water-solubilized suspension of carotene into a human subject Psoriasis patients showed lower than normal amounts of carotene and vitamin A within the sebaceous system after injection with the carotene suspension; patients with chronic ichthyosis and pityriasis rubra pilaris, diseases in which the sebaceous glands are often markedly atrophic or absent, showed no response (111) The authors believed that previous attempts to find vitamin A in skin and it appendages have failed because the vitamin normally exists within the sebum in reduced concentration or in modified form not detectable chemically.

The presence of 7-dehydrocholesterol (vitamin D_3 precursor) in human skin was demonstrated by Miller & Baumann in 1954 (112). This finding has been confirmed and extended by Wheatley & Reinertson (113), who found that the amount of the precursor in normal epidermis was approximately 12 times that in the dermis

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DELAYED SENSITIVITY AND HOMOGRAFT SENSITIVITY[1]

By H. Sherwood Lawrence, M.D.[2,3]
Department of Medicine, New York University College of Medicine, and the Third (NYU) Medical Division Bellevue Hospital, New York City, New York

It is now generally accepted that the rejection of foreign homologous tissues applied as homografts to genetically unrelated individuals of the same species is accomplished by an immunologic response undertaken by the host (20, 48, 63, 66, 80, 83 to 85, 102). However, it remains to be established whether the highly specific immune mechanism that results in tissue destruction is mediated by serum antibody of the classical type or by a factor or factors intimately bound to cells of the leucocyte series. The experimental resolution of this question has been under concentrated attack in recent years and its outcome is the focus of much interest. The prominence accorded this issue arises from the reality that the possibility of ameliorating or abolishing this acquired antagonism to another's tissues, an immune response possessed of exquisite specificity, is dependent upon the precise definition of the antigen(s) that induce it and the antibody(ies) that mediate it.

Since the more general biological problems encompassed by the study of homograft rejection phenomena have been considered at length in several recent reviews (20, 48, 66, 83 to 85, 102), the present discussion will be restricted to the evidence for homograft rejection as an actively acquired immune response and to a consideration of the immunologic mechanism or mechanisms by which this event may be accomplished.

It will be helpful to define here, as Brent, Brown & Medawar (22) recently have done, the exact type of homograft that will be under consideration. As they point out, the evidence recently summarized by Gorer (48) makes it unwise, if not impossible, to expect the same mechanism to be operative in the destruction of all types of homografts Gorer (48) has delineated three different varieties of homograft response: (*a*) that evoked by leucotic cells; (*b*) that evoked by ascites tumor cells; and (*c*) that evoked by organized tissues characterized by the establishment of vascular

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donor previously experienced by the host will undergo accelerated rejection and grafts from unrelated donors will enjoy the deceptive initially longer acceptance accorded to first-set grafts (13, 14, 76 to 79).

It has been established that it is the genetic disparity between donor and recipient of homografts that calls into being this exquisitely sensitive discriminatory apparatus (19, 72, 102). The tempo and intensity of the homograft reaction is also conditioned by the degree of genetic difference of the donor in relation to the recipient. In general, the greater the genetic difference between two individuals of the same species, the more rapid the tempo and greater the vigor of the homograft rejection process. At the one extreme there occurs the complete acceptance of homografts exchanged between uniovular twins by virtue of their genetic identity (29). The absence of homograft rejection in uniovular twins has permitted the recent successful transplantation of kidneys between such individuals (54). The reliability of this finding has also been exploited in recent medico-legal proceedings undertaken to establish such a relationship (75). At the other extreme there occurs the violent reaction to heterografts of tissues transplanted between individuals of different species (e g, rabbit to guinea pig). Here the genetic dissimilarity is so great and the consequent antagonism evoked in the host so intense, that the initial period of acceptance afforded the homograft is deprived the heterograft (73).

The homograft reaction, once instituted, is in force throughout the individual wherever tested and is generalized in its distribution (13, 14). It has also been shown to be conditioned by the dosage of tissue used to produce it Medawar (76, 77) demonstrated that the length of survival of a homograft varied inversely as the dose or quantity of tissue applied. He was able to increase the survival time of a skin graft by one-half when he reduced the quantity of skin applied by one-eighth.

Medawar (79) also demonstrated that the homograft rejection reaction is individual-specific rather than organ-specific. In the rabbit he showed that prior inoculation of leucocytes caused the recipient to react to a subsequent skin homograft from the leucocyte donor as if it had met and reacted against that donor's skin before

Thus, the exquisite specificity of the homograft reaction, the latent period for sensitization, the need for prior sensitization to induce accelerated rejection of subsequent homografts from the same donor, and the dosage phenomenon, all contribute to the establishment of the homograft rejection reaction as an actively acquired immune response undertaken by the host against genetically unrelated tissues Medawar (82, 83) has indicated that the immune response to foreign homologous tissue is actively acquired and does not pre-exist in the sense that individuals of blood group A possess natural agglutinins against the erythrocytes of all individuals of blood group B He cites the requisite latent period between contact with antigen and

and lymphatic connections with the host. As they have done (22), we would elect to restrict comparison between delayed allergy and homograft sensitivity to the most extensively studied example of the third type of homograft—the orthotopic skin homograft. The compelling reason for the selection of the skin homograft as an experimental test model derives from its function as a prototype. It is the type of target against which ultimately all practical efforts to abolish homograft sensitivity must be directed.

Before discussing mechanisms of homograft rejection it will be helpful to review very briefly the data upon which is based the conclusion that this untoward event is indeed an acquired immune response.

HOMOGRAFT SENSITIVITY—AN ACTIVELY ACQUIRED IMMUNE RESPONSE

When skin is excised from one individual and transplanted to a similar defect in the skin of another individual of the same species it is known as an *orthotopic homograft*. This having been accomplished under appropriate surgical circumstances, the homografted skin will remain bloodless for two or three days until it is invaded by blood vessels and lymphatics from the host to form *anastomoses* with the vessels and lymphatics of the graft. Soon thereafter, the host's blood may be seen to course through the vessels of the graft, nourishing it and ensuring its viability. This symbiotic relationship between host and new tenant remains placid and uneventful until the tenth to twelfth day following transplantation. At this time the blood flow through the vessels of the graft becomes sluggish, erythrocytes agglutinate into rouleaux, intravascular thrombi are formed, and subsequent capillary rupture with widespread hemorrhage into the graft occurs. The gross evidence of this microscopic disaster soon follows with progression to necrosis of the graft, eschar formation and, finally, rejection of the now scabrous foreign tissue (13 to 15, 30, 71, 76, 77, 97). In contrast, an *autograft* of the host's own skin, excised and transplanted to another area on his body, will establish itself as the homograft had done, with the difference that it is permanently accepted, and becomes again an integral part of the host (76).

The preceding description of the fate of a homograft applies only to the occasion of the initial meeting between host and a particular donor's tissues and has been termed a "first-set" reaction. The behavior of the same host toward a skin homograft transplanted from the same donor on a second occasion some weeks later is quite different. In this instance the untoward events described above are now set in motion on the fourth to sixth day and the graft is rejected in an accelerated fashion—the "second-set" reaction. The fact that this event registers a highly specific remembrance of prior contact with a particular donor's tissues, can be demonstrated by the application of multiple skin grafts to the same host from a variety of donors. In individuals so conditioned, only the graft from that

is detected when such sera interact with the erythrocytes of the tumor donor. The other, a neutralizing antibody, may be demonstrated by two variations of the same biologic test: (*a*) pre-incubation of tumor cells and immune serum before injection into the recipient; (*b*) transfer of immune serum at the time of or shortly before tumor inoculation. In each instance, the neutralizing antibody inhibits the growth of tumor cells The appearance of both hemagglutinating antibody and neutralizing antibody coincident with the rejection of transplanted tumor cells has suggested a causal relationship between the two events. However, as Gorer has shown (44, 48) the hemagglutinating antibody may be absorbed from sera containing both types of antibody by means of mouse erythrocytes, without effect upon the biological activity of the neutralizing antibody Further dissociation of the hemagglutinating antibody from the neutralizing antibody may be produced by pretreatment of the recipient with lyophilized tumor tissue, as Kaliss (56 to 58) has done This results in an opposite effect, namely "enhancement" of the growth of tumors subsequently transplanted, rather than rejection, despite—or perhaps because of [cf (48)]—the rising titer of hemagglutinating antibody. Mitchison (88 to 90) has also shown a temporal dissociation in onset of hemagglutinating antibody and the onset of homograft rejection in mice inoculated with sarcoma He found in specifically sensitized animals, that the capacity of cells to transfer accelerated graft rejection appeared several days before detectable hemagglutinating antibody. The latter also remained at peak titer when the capacity for cellular transfer of accelerated rejection had disappeared.

Medawar (83, 85) has clearly delineated the distinction between the antigens responsible for the production of hemagglutinating antibody (H-antigens) and those responsible for transplantation immunity (T-antigens) in the mouse. "H-antigens" resist heating and lyophilization, reside on the surface of erythrocytes and other cells, are restricted to the cytoplasm of nucleated cells, and appear later in embryological development of the mouse "T-antigens," in contrast, are destroyed by heat or freezing, have not been detected in erythrocytes, or in the cytoplasmic fractions of nucleated cells.

As indicated above it had been shown for mice by Kaliss (56 to 58) and recently for goldfish by Hildemann (52) that hemagglutinating and other serum antibodies may be present when homograft sensitivity is absent More recently, Hildemann & Medawar (53) have confirmed this finding for [illegible] the abse[illegible] tion of [illegible] demonstration that homograft sensitivity could be produced by crude splenic nuclear fractions without the production of hemagglutinins or hemolysins. It was further shown that such nuclear fractions were incapable of absorbing the humoral antibodies from sera

From the evidence available it appears very unlikely that hemagglutinat-

acquisition of sensitivity and the reality of the dosage phenomenon to support this distinction.

THE CHOICE OF IMMUNE MECHANISMS AS INSTRUMENTS OF HOMOGRAFT REJECTION

There are four potential immunological effector mechanisms any one or combination of which could be set in motion by the antigenic stimulus of foreign homologous tissue and result in destruction of the tissue concerned: (*a*) Homograft rejection may be mediated by serum antibody of the classical type with the mechanism of tissue damage analogous to that characteristic of Arthus sensitivity. (*b*) Homograft rejection may be mediated by a cell-bound immune factor or factors with the mechanism of tissue damage analogous to that characteristic of tuberculin sensitivity. (*c*) Homograft rejection may be mediated by a combination of effects instituted by serum antibody and cell-bound immune factors. (*d*) Homograft rejection may be mediated, as Thomas (106) has suggested, by an immune response peculiar to itself without precedent in conventional immunology and currently in the process of clarification. To the above possibilities we would add a variation—namely: (*b*-a) Delayed sensitivity and autoimmune disease may be expressions of homograft reactions undertaken by the host against certain of his own tissues.

Since the immunologic details and biological ramifications of delayed sensitivity and its relation to Arthus sensitivity and conventional humoral antibody responses have been considered extensively in several recent reviews (27, 28, 37, 62, 93), only those points relevant to the present argument will be touched upon here.

Evidence for Humoral Antibody as Effector of Homograft Sensitivity

Dissociated cell homografts—A substantial catalogue of evidence implicating serum antibody as the instrument of homograft destruction has been secured in relation to dissociated cell populations rather than solid orthotopic homografts Most studies have employed dissociated tumor cells characterized by rapid growth rates and perhaps a unique vulnerability therefore, to the antagonism afforded by serum antibody (5 to 7, 42 to 50, 56 to 58, 102)

The extensive evidence demonstrating the presence of serum antibodies following inoculation of tumor cell populations in the mouse has been collected and analyzed by Gorer and his associates (6, 7, 42 to 50). They have shown beyond doubt that certain tumor tissues of mice (leukemia, sarcoma) have the capacity to induce the production of at least two distinct types of circulating antibodies Neither antibody is demonstrable by conventional immunochemical techniques such as the precipitin or complement-fixation reactions One, a hemagglutinating antibody, appears in the sera of tumor-homografted mice coincident with the rejection of the graft. Its presence

of the host requires rapid multiplication of the microbe, as with tumor cells. The type-specific polysaccharide antigen responsible for the disease resides on the surface of the pneumococcus and it is to this locus that the humoral antibody is directed. The effects of specific antibody on the microbe result in capsular swelling as can be shown in the traditional Quellung reaction, an *in vitro* counterpart of *in vivo* events, which inhibits reproduction and ensures lysis of phagocytosed organisms.

We would therefore suggest the existence of an analogous relationship in tumor cell populations with the specific antigen residing at the surface of the cell and the intervention of neutralizing antibody at this locus. The effect of this interaction on the growth of the tumor cell appears to be qualitatively similar to the result of interaction with its specific antibody on the growth of the pneumococcus. The inhibition of tumor growth by serum antibody in this sense, suggests that the actual homograft rejection mechanism in this system, if sensitivity were induced without killing the mouse, could occur via delayed allergy This has been accomplished by Mitchison (86 to 90) with the cellular transfer of accelerated rejection of a solid tumor homograft (Sa 1) in mice as discussed below. It should be noted here, however, that he failed to demonstrate any effect of humoral antibody in passively transferred immune sera upon this type of solid tumor homograft. This finding fosters the conclusion that the unique vulnerability of populations of discrete cells to the adverse effects of humoral antibody is a function of their dissociated state.

Orthotopic skin homografts—The first attempts to transfer sensitivity to orthotopic skin homografts employed serum and yielded uniformly negative results The basic intent of this type of experiment is to cause the recipient to respond in the fashion of the donor who has been sensitized by a specific individual's tissues (i e, with a second-set reaction of accelerated rejection) Billingham, Brent & Medawar (14) prepared immune sera in inbred strains of mice sensitized with the tissues of the donor of the test homograft The use of inbred strains precluded any non-specific absorption of antibody from such sera They failed to transfer the capacity for accelerated graft rejection with small or large doses (up to 5 ml per mouse) of specific immune sera The recipients were injected with sera over a period of 3 days before and 6 days after the application of the test homograft. Peripheral blood from sensitized mice was also found to be ineffective

Billingham & Brent (11) later undertook an extensive restudy of this problem with similar negative results, despite the use of skin homografts rather than intact cells to sensitize serum donors. Serum was obtained from donors sensitized with first-set, second-set, or third-set grafts and inoculated intravenously, intraperitoneally, and subcutaneously into nonsensitive individuals without altering the behavior of the recipient to test homografts.

ing antibody is concerned with homograft destruction, even of tumor cells, but rather represents the expression of a common antigen shared between the fixed tissues (tumor, skin) and erythrocytes that are characteristic of certain strains of mice

However, the biological effects of neutralizing antibody on dissociated tumor cell populations have all the superficial appearances and behaviour of an antibody that could indeed function as the instrument of rejection of the transplanted tumor. Since the agents against which the antibody exhibits its antagonism are cells, their failure to grow has been interpreted as synonymous with rejection. In the animal undergoing primary stimulation with tumor cells, neutralizing antibody may be viewed in the broad sense as the cause of rejection of the cells that initiate its production. However, we would suggest that this potent humoral antibody does not function *to cause the accelerated rejection of tumor in the usual sense of that term* in the recipient of transfer, but rather it prevents the tumor from establishing itself initially by inhibiting its growth Evidence to support this interpretation is provided by Gorer's (48) data on the critical role played by time of administration in conditioning the effectiveness of serum transferred, in inhibiting growth of tumor cells in susceptible animals. He found that the intraperitoneal injection of serum containing neutralizing antibody suppressed the growth of tumor cells subsequently inoculated subcutaneously, when given as long as 7 days or as short as a few hours before challenge with tumor. However, when tumor inoculation was followed by immune serum 24 to 48 hr. later, the protective effect was less predictable.

Further evidence in support of this view is afforded by Gorer's (44, 48) finding that the neutralizing effects of immune sera incubated with tumor cells before the inoculation of the latter into a susceptible host, occurs very rapidly This finding gains significance when compared to the prolonged incubation (24 to 30 hr.) with immune serum obligatory for the demonstration of any effect on dissociated epidermal cells seeded back on to the donor sites (17, 18).

Gorer (48) and Woodruff (109) have suggested that humoral antibody in coating tumor cells may opsonize the latter and facilitate leucocytic action in synergistic fashion as antisera are known to opsonize bacteria

A clearer visualization of the distinction between mechanisms which preclude the initial "acceptance" of a homograft and those which cause rejection subsequent to a period of residence, may be gained from a consideration of events that follow pneumococcal infection (74) If a susceptible mouse is injected intraperitoneally with a Type I pneumococcus, rapid reproduction of the microbe ensues and the mouse succumbs to an overwhelming infection If, on the other hand, the mouse is given type-specific antiserum before the pneumococcal injection, the growth of the microbe is inhibited and the infection never gets under way. The pneumococcus has its own fastidious growth requirements and to cause infection and death

negative results. Their main criterion for sensitivity was the histological evidence of diminished test graft survival, in addition to the degree of vascularization They also attempted to cause rejection of established homografts in tolerant animals by repeated injection of anti-CBA serum into A strain mice bearing CBA grafts with negative results. Both Stetson and Brent, in a discussion of these results, call attention to the differing criteria used to detect successful transfer with serum as a possible explanation of the different results achieved [see (22)] Stetson, in this discussion, also reports on his failure to transfer the capacity for rejection of grafts to tolerant recipients of serum, and points out that he has found it difficult or impossible to affect established grafts in non-tolerant animals with immune sera.

The observations of Good and his colleagues (38 to 41), on the prolonged retention of skin homografts in two patients (2 years, 14 months) afflicted with congenital agammaglobulinemia has strengthened the interpretation that classical humoral antibody is required for homograft rejection. This interpretation is based on the demonstration that such patients, including the two under consideration, have the capacity to develop delayed allergy in the face of an inability to produce circulating antibody (40, 41, 95).

It is not known whether serum antibody is indeed indispensible for homograft rejection or whether prolonged retention of the grafts in these individuals is a parallel reflection of their profound deficiency in immunological mechanism. The case for serum antibody is weakened, as Medawar has indicated (81, 83, 85), by the observations of Schinckel & Ferguson (101) on fetal lambs. This species, despite a persistent natural agammaglobulinemic state until after birth, possess an unimpaired capacity to reject skin homografts.

The case for a parallel but unrelated deficiency of immunological mechanism, is suggested by recent observations made on patients with Hodgkin's disease and uremia. Kelly, Good & Varco (59) have reported the prolonged survival of skin homografts transplanted to patients with Hodgkin's disease The immunological defect in such patients is generally expressed by a loss of the capacity to manifest sensitivities of the delayed type coupled with an unimpaired capacity to produce circulating antibodies. In contrast to agammaglobulinemic individuals, patients with Hodgkin's disease usually exhibit hyperglobulinemia. Dammin *et al* (31) found prolonged survival of skin homografts in patients with chronic uremia who had normal gamma globulin and antibody levels, and were tuberculin positive as well.

Evidence For Cell-Bound Immune Factors as Effectors of Homograft Sensitivity

Since the similarities between tuberculin allergy and homograft sensitivity are multiple and course through the various phases of each immune

The strongest evidence that classical humoral antibody may play a role in the rejection of orthotopic skin homografts derives from the recent experiments of Stetson (103, 104). Mice immunized with spleen cells in Freund's complete adjuvant, were found to respond to a skin homograft from the spleen donor with a "white graft" reaction of rejection. Serum transferred from these animals caused recipients to respond to homografts of the sensitizing individual's skin with a "white graft" reaction, while homografts from unrelated individuals were accorded a first-set response. Similar experiments were performed in rabbits with comparable results.

Stetson has underlined clearly and precisely the fact that the response transferred by serum is a passive one and distinguishes it from the transfer of the mechanism of homograft rejection. He has stressed also the gap between the demonstration of serum to cause a "white graft" reaction, which he regards as a heightened, but as yet unknown, type of graft response, and the conclusion that humoral antibody is indeed the effector of homograft rejection.

Stetson's experiments differ from those of Billingham, Brent & Medawar (11, 14) referred to above in three significant aspects: (*a*) spleen cells in Freund's adjuvant were used to immunize serum donors; (*b*) this procedure resulted in a "white graft" reaction instead of accelerated rejection; (*c*) the expression of homograft sensitivity transferred was a "white graft" reaction

The immunological meaning of the "white graft" reaction is itself currently undergoing investigation and evaluation. This type of response to foreign skin is evoked when transplantation between individuals of different species is undertaken and it is characteristic of the primary response of non-sensitive animals to heterografts. In transplantation of skin homografts between individuals of the same species the "white graft" can be made to replace the usual response of accelerated rejection by shortening the interval between the initial, first-set reaction and the application of subsequent grafts This has been demonstrated in mice (14), rats (71), and man (96, 97). As a variation of the second-set response to skin transplanted to a specifically sensitized individual of the same species, it differs from accelerated rejection in the failure of initial vascularization of the graft before rejection occurs. The "white graft," in its demonstrable individual specificity and in its requisite for prior sensitization, represents a significant but as yet incompletely understood manifestation of homograft sensitivity. It may be seen eventually as a manifestation of a more intense and heightened state of homograft sensitivity than accelerated rejection as suggested by Stetson (103, 104) and it could be brought about by an Arthus reaction occurring in the capillary buds of the graft, as suggested by Gell (104)

Brent, Brown & Medawar (22) have recently used Stetson's experimental design for attempts at serum transfer of homograft sensitivity with

negative results. Their main criterion for sensitivity was the histological evidence of diminished test graft survival, in addition to the degree of vascularization. They also attempted to cause rejection of established homografts in tolerant animals by repeated injection of anti-CBA serum into A strain mice bearing CBA grafts with negative results Both Stetson and Brent, in a discussion of these results, call attention to the differing criteria used to detect successful transfer with serum as a possible explanation of the different results achieved [see (22)]. Stetson, in this discussion, also reports on his failure to transfer the capacity for rejection of grafts to tolerant recipients of serum, and points out that he has found it difficult or impossible to affect established grafts in non-tolerant animals with immune sera.

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Since the similarities between tuberculin allergy and homograft sensitivity are multiple and course through the various phases of each immune

response, it will be helpful to consider each phase under a separate heading.

Induction of sensitivity.—A similarity between the living agents that induce tuberculin sensitivity and those that induce skin homograft sensitivity is suggested from the fact that both are composed of fairly uniform populations of slowly dividing cells which, usually, do not overwhelm the host Both cell types share in common a characteristic complexity of structure which results in intricately arranged aggregates of antigens, each capable of provoking an immune response directed against a particular stereo-specific site In tuberculous infections, serum antibody directed against the polysaccharide antigen can be demonstrated, yet the tissue damage incurred has been shown to occur in the absence of serum antibody and to be the exclusive property of delayed allergy to tuberculin (9, 34, 36, 99). We have discussed earlier the occurrence of hemagglutinating antibody following sensitization to skin homografts, and the demonstration that homograft rejection can occur in the absence of this antibody (53).

The parallelism is furthered by the finding that extracted products of the tubercle bacillus are capable of evoking serum antibodies and anaphylactic sensitivity quite unlike the state of delayed sensitivity produced by the intact bacillus This response is distinct and separable from the induction of delayed allergy and is a testimonial to the fact that extracted products of the tubercle bacillus are potent antigens (99, 100). A somewhat analogous situation is produced when intact tissue cells (tumor, skin) are extracted by lyophilization. Whereas the intact cells produce homograft sensitivity, the extracted products produce its opposite "enhancement," a reversal of response also associated with, and like, anaphylactic sensitivity, dependent upon high titers of serum antibody (56 to 58)

The route of sensitization employed also conditions the effects achieved with bacterial cells or with tissue cells In both instances the intravenous administration of either cell type fails to produce sensitivity. It has been shown for the tubercle bacillus, streptococcus, and pneumococcus that intravenous administration does not result in delayed allergy to the specific microbe but in a state of immunity to infection. To produce delayed allergy, it was found to be obligatory that intact microbial cells be administered subcutaneously, intradermally, or intraperitoneally (32, 55, 94, 105).

Medawar (79) was the first to observe that the production of homograft sensitivity depended upon the route of administration of the tissue cells used as sensitizers He sensitized rabbits with leucocytes and studied their response to subsequent skin grafts from the leucocyte donor. In this study it was observed that the intradermal injection of sensitizing leucocytes was 18 times more effective than the intravenous route This finding was later confirmed and extended by Billingham & Sparrow (18) who substituted dissociated epidermal cells of the prospective skin graft donor rabbit to sensitize recipients They found that the intravenous route was not only

incapable of inducing homograft sensitivity (accelerated rejection) but produced an opposite effect in the prolonged survival of subsequent skin grafts. This effect was shown to be specific for the epidermal cells related to the test graft. The efficacy of the intraperitoneal and subcutaneous routes in the production of homograft sensitivity by this means was also confirmed.

This sort of finding both for bacterial and tissue cells suggests that the access of an inducing agent to a particular population of reticuloendothelial elements is a requisite for the development of delayed sensitivity. Another interpretation could view the effects achieved by the intravenous route as the result of sensitization and desensitization occurring *pari passu*. Some support for the latter view is afforded by the clinical observation of disappearance of tuberculin sensitivity in patients with tuberculosis at the time of blood stream invasion by the tubercle bacillus (99, 100).

Manifestations of Sensitivity

The Koch phenomenon and accelerated rejection—On a previous occasion (63), we have suggested that homograft sensitivity in its acquisition and manifestations bears a strong resemblance to the Koch phenomenon observed in animals infected with the tubercle bacillus.

In the non-sensitive animal, the initial bland acceptance of the tubercle bacillus, like the acceptance of the skin homograft, after a latent period of 10 to 14 days is superseded by an inflammatory response resulting in one instance in the rejection of tubercle bacilli and host tissue, and in the other in the rejection of the graft In each situation, this antagonism to the specific foreign cells is expressed with greater force and rapidity where the inflammatory sequence, now telescoped, produces the same result The primary event was thought to represent the acquisition of sensitivity to skin homografts (first-set reaction) and the secondary event to represent the manifestation of actively acquired sensitivity (accelerated rejection) on the basis of timing, specificity, and the requirement for prior sensitization.

The "recall flare" reaction—Support for the above view may be found in the "recall flare" reaction observed in humans by Rapaport & Converse (96, 97) The "recall flare" is an erythematous, indurated, inflammatory reaction which occurs in quiescent sites of previously rejected skin homografts coincident with the accelerated rejection of subsequent skin grafts from the same, but not from other donors The temporal course of this reaction is delayed in type, with onset occurring 24 hr after accelerated rejection of the specific graft is begun and subsiding at 48 hr. It has been likened to a similar flare-up of quiescent depots of streptococci in rabbit skin described by Andrews, Derick, & Swift (8) and considered by them as a "Koch phenomenon" provoked by the acquisition of delayed sensitivity to the streptococcus.

Medawar and his colleagues (unpublished data) have recently observed the "recall flare" in healed quiescent sites of rejected skin homografts in

guinea pigs undergoing intensive hyperimmunization with leucocytes from the skin donor. It has similar characteristics as the reaction described in human subjects.

The "recall flare" reaction may be said to represent a delayed type of allergic inflammatory response to residual bacterial or tissue antigens in specifically sensitized individuals in the process of active sensitization. As such it strengthens the analogy drawn between the Koch phenomenon and homograft sensitivity described above.

The delayed skin test to tissue antigens and the tuberculin reaction—One of the impediments to a general acceptance of homograft sensitivity as a manifestation of tuberculin type sensitivity has arisen from technical differences inherent in the demonstration of the former response. Despite other data supporting a similarity between the two responses, a degree of tempered enthusiasm was required to visualize, in the act of accelerated rejection of a piece of skin, the operation of the same machinery that results in a delayed cutaneous reaction to tuberculin. This operational deficiency has been remedied recently by Brent, Brown & Medawar (21, 22) with the perfection of an intradermal test which reveals the presence of homograft sensitivity and is equated with accelerated rejection. In principle, a recipient guinea pig (R) is sensitized to skin homografts from the donor guinea pig (D). At the height of the graft rejection period (12 days) an intradermal test of (R) is performed with extracts of spleen or lymph node cells (antigen) prepared from (D). The sensitized animal (R) responds with a delayed tuberculin-like inflammatory reaction to the antigenic extract. The positive cutaneous reaction has its onset at 5 hr. and reaches its maximum intensity at 24 to 48 hr. and on histological examination exhibits the classical characteristics of the tuberculin reaction. This response has been termed the "direct reaction" to distinguish it from the "transfer reaction" described by the authors and considered below. Living donor cells are as effective in eliciting the "direct reaction" as extracts of cells. The "direct reaction" has its specificity directed only against the antigens prepared from tissue of the particular sensitizing donor (D) and does not cross-react with antigens prepared from tissues of other individuals

The authors conclude that the "direct reaction" depends on a state of sensitivity specifically directed against the antigens used in the skin test In this sense, the antigenic extract of tissues used as the test material and the response evoked has a strong resemblance to the reaction observed in tuberculin-sensitive animals tested with an extract of tubercle bacilli. Both materials have the capacity to indicate by a positive skin reaction a specific state of acquired delayed sensitivity

Cellular responses to homografts in diffusion chambers—The ingenious and painstaking studies of Algire and his colleagues (1 to 4, 108) have done much to clarify the role of cell-bound immune factors in the intimate

aspects of homograft rejection. They devised a technique whereby the target graft, placed within chambers of graded porosity, can be observed after transplantation intraperitoneally to specifically sensitized mice. By altering the chamber pore size, the host's cellular elements may be permitted or denied access to the interior of the chamber. Under all circumstances, materials in solution are freely diffusible, passing in and out of the chamber without impediment. In the sensitized animal, unprotected target grafts were rapidly destroyed (3 days) while identical grafts protected by chambers impervious to the host's cellular elements survived for prolonged periods (11 to 21 days, 4 to 6 months). In a similar situation, target grafts were placed in two types of chamber—one permeable and one impermeable to leucocytes and macrophages. The grafts in cell-permeable chambers were invaded and destroyed by host leucocytes and macrophages, while the grafts in cell-impermeable chambers survived and remained viable. This result occurred despite the demonstration that gamma globulin, diphtheria antitoxin, cytotoxic antibody, and mouse antihemolysins could be shown to pass freely into this type of chamber impermeable to host cells Histologic studies revealed a large variety of cells invaded in the cell-permeable chambers, but not all participated in graft destruction The authors conclude that the cells engaged in graft destruction are lymphocytes and the actual process of destruction does not entail phagocytosis.

These results were reinforced when target grafts were placed in cell-impermeable chambers and transplanted to isologous mice, excluding any possibility of the host reacting against the graft. To the test chamber was added spleen cells from mice sensitized to target graft and to the control chamber was added an equivalent volume of spleen cells from nonsensitive mice. Only the grafts in the chambers containing sensitized spleen cells were destroyed and rejected, while the grafts in chambers containing nonsensitive spleen cells survived unscathed. This series of experiments has secured an array of cogent evidence to demonstrate that homograft destruction, in these circumstances, occurred following interaction with sensitized cells alone in the absence of any possible humoral factor.

Transfer of Homograft Sensitivity by Means of Cells and Cell Extracts

The most significant and distinctive property characteristic of delayed sensitivity is the uniform susceptibility of this response to transfer by cells of the leucocyte series. The factual evidence for this statement and the broader biological implications of the cellular transfer system have been considered extensively in several recent reviews (26 to 28, 62, 64, 65, 67, 92). For the purpose of the present discussion it is sufficient to indicate that a wide variety of delayed sensitivities occurring in animals (24 to 28) and in man (60 to 62, 64, 67 to 69), have been transferred by means of cells of the leucocyte series (e.g, delayed sensitivity to bacteria, fungi,

virus particles, simple chemicals, nervous tissues, and specific precipitates). This unique property of delayed sensitivity gains force from the failure of serum to transfer such responses and the failure of cells to transfer Arthus sensitivity (37, 107), despite the ease with which the capacity for serum antibody formation may be transferred by cells (25, 26, 51).

Mitchison (86 to 90) was the first to demonstrate that homograft sensitivity (accelerated homograft rejection) is also transferred by means of cells. His use of inbred strains of mice excluded the possibility of a homograft reaction to the transferred cells The essential features gleaned from this series of studies, using solid tumor (lymphosarcoma) to sensitize, were as follows: the capacity for transfer was restricted to cells obtained from draining lymph nodes (contralateral nodes, whole blood, spleen, and peritoneal exudates, as well as serum were ineffective) of sensitized animals; it endured for three weeks after primary sensitization; and the cells concerned were inactivated by freezing and thawing When the temporal relationship between the appearance of hemagglutinating antibody and homograft rejection was studied in sensitized donors and recipients of cells, a dissociation of the two events was observed. In the actively sensitized animal hemagglutinating titers reached a peak 5 days after a first-set homograft had been rejected and 2 days after the second-set rejection. Similarly, the capacity of cells to transfer each response was dissociated in the recipient The capacity to transfer hemagglutinin production occurred later after sensitization (14 days) than the capacity to transfer homograft rejection (6 days). Mitchison (90) concludes from these studies that the capacity for hemagglutinin production and the capacity for homograft rejection are separable functions of the antigenic stimulus both in the actively sensitized animal and the recipient of cell transfer. He regards the two functions measured as parallel but unrelated events and favors the interpretation that homograft rejection occurs via cell-bound antibody analogous to delayed sensitivity of the bacterial or contact chemical type.

The cellular transfer technique was next applied to an analysis of skin homograft rejection in mice with similar success by Billingham, Brent & Medawar (14) The authors also used inbred strains (CBA) of mice for donor and recipient pairs, the donor differing from the recipient only by the presence of the immune factor (directed against A strain skin) carried by the sensitized leucocytes. Cellular transfer caused the recipient to behave like the sensitized donor to A strain skin, resulting in accelerated rejection The effective cells, as in Mitchison's findings, were restricted to the lymph nodes draining the grafted area, or at furthest point and to a lesser extent, the spleen. Also, in confirmation of Mitchison's results, whole blood, concentrates of blood leucocytes, and serum were ineffective and the capacity of effective cells was inactivated by grinding in a mortar.

An even more exacting demonstration of this exclusive property of cells was subsequently afforded by the transfer of homograft sensitivity to

tolerant mice by Billingham, Brent & Medawar (15). The CBA strain recipients of transfer had tolerated A strain skin grafts as autografts for prolonged periods before transfer. When lymph node cells obtained from a CBA animal sensitized to A strain skin were transferred to such tolerant recipients, the A strain skin graft in 3 to 4 days began to undergo prompt rejection of the accelerated type. The identical experiment when repeated in other tolerant mice, but using normal lymph node cells from a nonsensitive CBA donor for transfer, resulted in a long latent period (15 days) before rejection of the A strain graft.

This difference in temporal response confirms the notion that cells from sensitized donors are primed to react against the target tissue and need no instruction, whereas cells from non-sensitive donors require sensitization before a response can be effected. This type of result has led the authors to designate the homograft response following cell transfer as "adoptively acquired immunity" They make the distinction that recipients of cell transfer differ from passively sensitized animals (i e, via preformed antibody) in their capacity to give a secondary response and from actively sensitized animals in never having given a primary response.

One of the more provocative findings the authors discuss relates to the continual shedding of antigens by the tolerated skin homograft, as revealed by active sensitization of non-sensitive cells upon transfer The likelihood that normal tissues are constantly so engaged, makes it imperative that the individual recognize such antigens as "self" to prevent the rejection of one's own tissues in like manner. How delayed sensitivity of the tuberculin type itself may bring about an aberration in self-recognition will be discussed below.

The most recent work done by Brent, Brown & Medawar (21, 22) that resulted in the development of a skin test ("direct reaction") for homograft sensitivity, has also shown that this delayed cutaneous reaction to transplantation antigens can be transferred by cells obtained from sensitive donors ("transfer reaction") The "transfer reaction" is produced by draining lymph node cells obtained from animals sensitized by skin homografts upon intradermal injection back into the donor of the skin graft By this maneuver the donor tissues, comprising a mass of antigens, interact with the sensitized leucocytes bearing the immune factor with specificity directed against that particular donor tissue, to result in a delayed cutaneous inflammatory reaction

The "transfer reaction" reaches a peak later (at 48 hr) and endures longer than the "direct reaction" The concentration of immune factor in the presence of a large residue of antigen may account for the greater intensity of the "transfer reaction" compared to the "direct reaction" where the concentration of reactants is reversed

The reaction has been shown to be specific for the tissues of the individual used to sensitize; heating cells capable of transfer (48 5°C for

20 min.) inactivates this property and the reaction cannot be transferred by serum obtained from sensitive animals. Differing from earlier studies (14, 90), contralateral lymph node cells were observed to produce reactions as strong as draining lymph node cells, probably an expression of the heightened level of discrimination of the test system.

The authors conclude both the "direct reaction" and the "transfer reaction" are manifestations of interaction between antigen and sensitized lymphoid cells. They point out that the demonstration of delayed cutaneous sensitivity to homograft antigens and its transfer by specifically sensitized cells and not serum, strengthens the analogy so frequently drawn between homograft reactions and delayed sensitivity to bacteria and simple chemicals.

A telling corollary of this type of experimental result has been achieved in mice by Berrian & Brent (10). Here, advantage was taken of the fact that tissue cells (spleen) can be prepared as antigenic extracts with full capacity to induce homograft sensitivity actively (16). Intact lymph node cells, obtained from mice sensitized to X mouse skin, were incubated in the presence of antigenic extract of X mouse spleen. Following incubation, the intact cells were centrifuged and the supernatant extract tested for its capacity to induce actively homograft sensitivity. It was found that antigenic extracts incubated with the specifically sensitized cells suffered an appreciable loss of capacity to induce homograft sensitivity, whereas treatment of antigenic extracts with non-sensitive cells did not alter the antigenic activity. The authors interpret this result to indicate specific and selective absorption of the antigen(s) responsible for homograft sensitivity by antibody-like reactive sites created in or at the surface of sensitive lymphoid cells It is postulated that such reactive sites are formed in the process of sensitization and play a dominant role in the destruction of the homograft to which specificity is directed The role of serum antibody in this process, if any, is viewed as a subsidiary one

The most recent successful application of the cellular transfer system to the analysis of mechanisms of homograft sensitivity has been made in human beings (70) It was found that skin homograft sensitivity (accelerated rejection) was transferred to non-sensitive human recipients by means of DNase-treated leucocyte extracts obtained from the peripheral blood of adequately sensitized donors The technique utilized involves repeated transplantation of skin homografts from subject (A) to subject (B), the prospective donor of anti-A leucocytes Extracts of sensitive leucocytes are injected into the shoulder of recipient (C) and cause the latter to respond with accelerated rejection to a test graft from (A) while a control graft from (D) is accorded a first-set response. In these experiments, as had been known for the transfer of delayed bacterial (35, 61, 64, 68) and fungal (98) sensitivity with leucocyte extracts in man, the degree of sensitivity of the donor plays a critical role in conditioning the success and intensity of transfer. For example, one homograft exposure was insufficient to

sensitize donor leucocytes to transfer systemic sensitivity (i e, extracts injected into the shoulder, test and control grafts applied to the forearm). Two successive homograft exposures (a first-set and second-set response) were insufficient to sensitize donor leucocytes to transfer systemic sensitivity, but were sufficient to transfer local sensitivity (i e, leucocyte extracts injected into the recipient in concentric, halo-fashion adjacent to test and control grafts). When, however, four successive homograft exposures (first-set, second-set, third-set, and fourth-set) were used to sensitize leucocyte donors, the transfer of systemic homograft sensitivity was readily effected. Neither serum from sensitized donors nor leucocytes from non-sensitive donors were found to be capable of transferring homograft sensitivity. No attempt was made to measure serum antibody in recipients of transferred sensitivity in view of the negative attempts at serum transfer and the demonstrated failure of peripheral blood leucocytes (40, 41) or leucocyte extracts (68) to transfer the capacity for serum antibody production in humans

The results of transfer of homograft sensitivity with leucocyte extracts generally parallel the results obtained from similar transfer of bacterial (35, 61, 64, 68) and fungal (98) sensitivity of the delayed type in man The observations are interpreted to fulfill a judiciously formulated criterion relating delayed sensitivity of the tuberculin type to homograft sensitivity. In addition, the successful use of leucocyte extracts in the transfer of homograft sensitivity affords an opportunity to attempt identification of the factor or factors (transfer factor) in sensitive human leucocytes concerned and analysis of the mechanism whereby this is accomplished. A step in this direction has been taken regarding delayed sensitivity to microbial products (61, 64, 68, 69, 98) and some of the information secured with the latter test systems has been applied successfully to the analysis of homograft sensitivity summarized above Although the precise biochemical or immunological nature of the transfer factor has yet to be defined, certain properties of the biologically active material have been described which suggest that this goal, although difficult, is not an impossible one. Since the biological and immunological properties of transfer factor in relation to delayed microbial sensitivity have been reviewed in detail elsewhere (64, 65, 67, 91, 92) this material will not be recapitulated here.

Of further interest to the problem of homograft sensitivity and its relation to delayed sensitivity, is the fact that differences in the behaviour of the cellular transfer system in each have been recently resolved Medawar (85), in an earlier review, pointed out the restriction of cells capable of transferring homograft sensitivity to lymph nodes draining the grafted area or, at furthest point, the spleen, departed from the experience with other delayed sensitivities wherein cells from lymph nodes, spleen, blood, or peritoneal exudates possessed the capacity to transfer sensitivity. Subsequent studies by Brent, Brown & Medawar (21, 22) in the guinea pig indi-

cated that cells obtained from contralateral lymph nodes in sensitive animals were as effective as cells obtained from draining lymph nodes. More recently, unpublished work in Medawar's laboratory has demonstrated that intact peripheral blood leucocytes are as effective as draining lymph node cells in producing the "transfer reaction" in the guinea pig This result, in an animal with the capacity to develop delayed sensitivity as a species characteristic and the capacity of extracts of peripheral blood leucocytes to transfer homograft sensitivity in human subjects described above, has clarified this problem. It would appear that the differences in behaviour of the cellular transfer system in homograft reactions arise from the degree of sensitivity possible for various species (mouse, guinea pig, man) to achieve, and the means of immunological expression at the disposal of each.

HOMOGRAFT SENSITIVITY AS AN IMMUNE RESPONSE UNIQUE TO ITSELF

Thomas (106) has suggested that the immune mechanism inadvertently uncovered by the inefficient attempts of mammals to eradicate bacterial infections, and revealed by the rather unusual circumstance of one individual *transplanting skin to another (a circumstance difficult to attribute* to evolutionary anticipation), evolved for a different purpose altogether. He postulates that the aggregation of cells forming multicellular organisms resulted in the evolution of a mechanism to recognize as foreign and to destroy mutant cells derived from such populations This mechanism *acknowledges mutant cells as an inevitable accompaniment of organized* cellular populations; cells that, in fact, only infrequently overwhelm the community of cells in which the mutation occurs Thomas suggests that the primary purpose of the mechanism which is inefficiently utilized to eradicate bacteria or foreign tissue, may have evolved to prevent neoplasia *The recent general experience that cell lines from normal individuals* maintained in culture for any period assume neoplastic characteristics, is cited in support of this notion. Thomas would therefore prefer to view the homograft reaction as a type of immune response peculiar to itself, but of the same genre as that utilized by the host to deal with bacteria, which is still *in the process of definition.*

DELAYED SENSITIVITY AS AN EXPRESSION OF HOMOGRAFT SENSITIVITY

We have postulated recently (66) that the delayed type of allergic *inflammatory response* may *itself* be a local type of homograft rejection, inadvertently undertaken by the host against his own tissues. The (self + X) hypothesis has been stimulated by the views expressed by Thomas (106) and the unique behavior of transfer factor responsible for delayed sensitivity in human species (61, 64, 68, 69, 92), and is based on the following facts (*a*) the agents which result in intense and durable

states of delayed sensitivity are characterized by prolonged intracellular residence in the host—preferential for the tubercle bacillus, obligatory for virus particles, and demonstrated for simple chemicals (33); (*b*) the reality of the "self-marker" theorem of Burnet & Fenner (23) as demonstrated by Billingham, Brent & Medawar (15) in the production of actively acquired tolerance upon exposure to another's tissues in embryonic life. The assumption required relates to the formation of intimate complexes between the intracellular components of the host (self) and the inducing agent (X). Although not yet shown for bacteria or viruses, the combination with host tissues has been shown to be obligatory for the induction of delayed sensitivity to simple chemicals (33). Mitchison (87) was the first to call attention to the similarity between a haptene-altered body protein complex and an *iso*-antigen and suggest an analogy between homograft sensitivity and contact sensitivity to chemicals.

The induction of delayed sensitivity is postulated as occurring when dead and dying macrophages bearing (self + X) complexes upon phagocytosis by host reticuloendothelial elements, are recognized as slightly foreign and as something other than (self). This event, partially out of the ordinary for the self-recognition system, induces the formation of an immune factor with specificity directed not against (self) alone or (X) alone, but against the (self + X) complex. It is suggested that this effector agent, a highly specific reactor site residing in or at the surface of sensitive cells of the leucocyte series, may be the "transfer factor."

The manifestations of delayed sensitivity are thought to occur wherever and whenever the inducing agent or its products (X) are in combination with host tissues (self) In its simplest form, this may be visualized in the intradermal tuberculin test where epidermal cells, ordinarily recognized as (self) upon being coated with (X), are recognised as a local group of (self + X) complexes Specifically sensitized leucocytes bearing the transfer factor seek out and attempt to destroy the local group of cells—an act viewed as a type of homograft rejection involving the host's own tissues. The similarity to homograft rejection is unmistakable in the Koch phenomenon in animals and in cavitary tuberculosis in human beings. In these situations the host, in attempting to reject the tubercle bacillus, inadvertently rejects his own tissues as well.

In an effort to interpret the behaviour of the transfer factor in human species, it has been suggested that the donor transfer factor is taken up by recipient macrophages to give the early expression of transferred sensitivity As an alternative to the proposals of a self-replicating or transducing agent to account for the prolonged duration of sensitivity following transfer in humans (61, 64), it is suggested that the combination (donor transfer factor + X) may substitute for the tubercle bacillus in the formation of a (self + X) complex in the recipient, causing the latter to undertake the production of his own transfer factor.

In this interpretation, the (self + X) complex, in causing the induction *and manifestation of a local type of homograft reaction* by the host against his own tissues, is not necessarily regarded as intense a stimulus as foreign tissues may be. Although proposed with delayed sensitivity of the bacterial, viral, or simple chemical type as models (66), this concept has been extended to include autoimmune disease (67). Allergic encephalomyelitis affords an explicit example where the virus infection or virus immunization may function as (X) in the production of the naturally occurring disease in man. In this example, the individual's nervous tissue supplies the (self) required and, in reacting against the (self + X) complex, portions of the individual's own nervous tissue are rejected in the fashion of a homograft. In the experimental disease produced in animals, the tubercle bacillus in Freund's adjuvant upon combination with host nervous tissue may provide a more predictable (X) as substitute for the virus of the naturally occurring disease.

The (self + X) hypothesis in the context discussed is founded upon the reality of the immunologic origins and consequences of individuality and should prove therefore susceptible of experimental attack.

CONCLUSIONS

In attempting to assess the immunological mechanism of homograft rejection, an effort has been made to evaluate the data in relation to the type of homograft studied. Humoral antibody, as an effector of homograft rejection, is most potent against dissociated tumor cell homografts. It is suggested that this unique vulnerability is a function of the dissociated state and rapid growth rate inherent in that type of homograft. On the other hand, delayed sensitivity and cell-bound immune factors appear to play a predominant role in the rejection of solid homografts. There is no single piece of experimental evidence which makes this conclusion an established fact. However, considered as an integrated whole, the isolated pieces of information form a continuum preponderantly in favor of this conclusion.

Nevertheless, a mutually exclusive selection of delayed sensitivity over humoral antibody, or vice versa, is not warranted by the facts currently available, nor would such a selection serve any useful purpose. Humoral antibody may play a role in the rejection of orthotopic skin grafts, despite the difficulties encountered to date in its detection. From the evidence reviewed, however, it would appear that the role would be a subsidiary one.

It is not without interest that the problem of identification of the specific antigen(s) and characterization of the specific antibody(ies) responsible for homograft sensitivity would be greatly facilitated if the effective antibody were detectable in the serum. The situation at present, however, is such that the nearest facsimile to an antibody system for the analysis of homograft sensitivity, as for delayed sensitivity, is that afforded by cellular

transfer. This system alone, in addition to bearing the immune factor or factors which set in motion the events that lead to homograft rejection, also contains the mechanism which produces that immune response. These effects are dissociated in the temporal difference in homograft rejection in tolerant animals confronted with nonsensitive cells as compared to sensitive cells, and in the transfer of homograft sensitivity with leucocyte extracts in human beings.

How homograft sensitivity has come to be viewed as a variant of delayed sensitivity and how delayed sensitivity may be viewed as a variant of homograft sensitivity has been discussed. That such views need not be mutually exclusive; that they offer interesting ramifications; and that they may suggest new approaches to specific problems, has been indicated

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THE L.E. CELL PHENOMENON[1,2]

By Halsted R. Holman, M.D.
The Rockefeller Institute, New York, New York

The LE cell, which appears in the blood of patients with systemic lupus erythematosus (SLE), has proved of interest in two major respects. First, study of the nature of the cell has led to the identification of a group of antibodies to constituents of the cell nucleus, including deoxyribonucleic acid. This constitutes the first example in human disease of an immunological reaction involving the nucleus. Secondly, because these antibodies can react with nuclear materials from the patient, they may be considered autoantibodies and they add to the growing number of autoimmune reactions identified in SLE. One of these antibodies appears to be responsible for formation of the LE cell. Thus, the LE cell is the product of an autoimmune reaction involving the cell nucleus.

MORPHOLOGICAL AND HISTOCHEMICAL STUDIES

The LE cell was first described by Hargraves *et al.* (1). It appears in the drawn blood of patients with SLE after that blood has been allowed to stand for a matter of minutes. Haserick *et al.* demonstrated that the LE cell results from the action upon leukocytes of a substance in the patient's serum which migrates on electrophoresis with the γ-globulin (2). Subsequent studies have identified the steps involved in the formation of LE cells (3, 4). Initially, the nuclei of certain leukocytes swell and become homogeneous These altered nuclei are then extruded from the cell and are phagocytized by other viable leukocytes. The latter leukocytes, containing the altered nuclei in their cytoplasm, are the LE cells Three ingredients are necessary for this reaction: the serum factor (LE cell factor), the nuclei to be altered, and leukocytes capable of phagocytosis.

Initial studies of this phenomenon were conducted by histochemical methods. Evidence was obtained which was interpreted as demonstrating that depolymerization of deoxyribonucleic acid (DNA) is the primary event in the alteration of the nucleus (5). It was inferred that the serum factor activated an intracellular deoxyribonuclease which in turn caused the depolymerization (6). Recent detailed histochemical investigations by

[1] The survey of the literature pertaining to this review was concluded in June, 1959

[2] The following abbreviations will be used· DNA (deoxyribonucleic acid); DNase (deoxyribonuclease); LE (or L.E.) lupus erythematosus); SLE (systemic lupus erythematosus).

Godman and his associates do not support these conclusions (7). They have demonstrated that depolymerization does not occur. Instead, the initial swelling of the cell nucleus is accompanied by a striking rise in intranuclear protein. They suggest that the LE cell factor induces its effect by direct combination with a constituent of the nucleus, probably the DNA.

IMMUNOLOGICAL STUDIES

Antinuclear antibodies.—Initial impetus to the study of the LE cell by immunological methods was given by Miescher who observed that the passage of isolated cell nuclei through serum from patients with SLE removed or diminished the ability of this serum to cause formation of LE cells (8). Subsequent investigations have shown that the LE cell factor reacts with whole cell nuclei and also with isolated nuclear deoxyribonucleoprotein, which consists primarily of DNA and the basic protein histone in salt linkage (9 to 12). Nucleoprotein quantitatively absorbs the LE cell factor and, once coated with it, is phagocytized with formation of cells resembling LE cells Removal of either DNA or histone from the nucleoprotein abolishes the reaction, as does substitution of hyaluronic acid or polyethylene sulfonic acid for the DNA or substitution of protamine for the histone. Thus, both DNA and histone are essential for the reaction Nucleoprotein appears to be the constituent of the nucleus with which the LE cell factor specifically reacts (13). Interestingly, prior exposure of nucleoprotein to quinacrine hydrochloride (Atabrine Dihydrochloride), which has some therapeutic effect in SLE, prevents subsequent reaction of the nucleoprotein with the LE cell factor.

The LE cell factor is not the only factor present in SLE serum which reacts with the constituent of the nucleus. Other factors have been identified which react with isolated DNA, purified histone, and another unidentified substance which can be removed from the nucleus by extraction with 0.1 ionic strength buffers (14, 15) These serum factors have been demonstrated by the techniques of complement-fixation (16 to 19), red cell or latex particle agglutination (20 to 22), precipitation (23, 24), and passive cutaneous anaphylaxis (25) The factor which reacts with DNA yields precipitin curves when mixed with increasing concentrations of DNA, and forms precipitin bands with DNA in agar. The nature of the antigen extracted from nuclei with 0 1 ionic strength buffers is unknown, but it is neither DNA nor histone. The antinuclear factors do not demonstrate either species or organ specificity; nuclear constituents isolated from various tissues of animals and fish and from microorganisms have been reactive.

The different antinuclear factors can be separated one from another by absorption of serum with nuclear constituents and by recovery from the complexes formed with these constituents All, some, or none of the factors may be present in any individual serum In general, the LE cell factor and

the factor which reacts with the buffer extract of nuclei occur most frequently. The factor which precipitates with isolated DNA is less common and the factor which reacts with histone alone is rare, at least as identified by currently utilized techniques (15).

All of the antinuclear factors migrate with the γ-globulin on electrophoresis and sediment with the γ-globulin on ultracentrifugation Two of the factors have been isolated: the LE cell factor (13) and the factor which precipitates with DNA (23, 26). The former may be obtained from its complex with nucleoprotein by digestion of the complex with deoxyribonuclease (DNase) followed by elution from the residue at 56°C or at higher salt concentrations. The latter can be obtained by DNase digestion of the equivalence point precipitates from mixtures of SLE serum and DNA. In each instance, the only serum protein recovered has been γ-globulin. These γ-globulins have reacted completely with and been inactivated by antiserum to normal human γ-globulin The LE cell factor is stable at pH 2 and pH II for 24 hr. and is inactivated at temperatures above 65°C.

Four general types of methods may be used to demonstrate antinuclear factors The first consists of identification of antibody γ-globulin adherent to isolated nuclear constituents or to cell nuclei in tissue slices after they have been exposed to SLE serum. This can be accomplished by (*a*) reacting the test material with a labelled antibody to γ-globulin and observing the localization of the label (12, 27 to 30), or (*b*) by reacting the test material with an antiglobulin serum of known titer and measuring the drop in the titer after the reaction (31) An example of the former is the fluorescent antibody method The latter is known as the γ-globulin or Coombs consumption method These are probably the most sensitive techniques available for detecting the localization of γ-globulin on nuclei However, because they identify all antibody γ-globulin, they do not distinguish between the different antinuclear reactions. The second method consists of direct identification of an antigen-antibody reaction by fixation of complement, precipitation, or passive cutaneous anaphylaxis All of the antinuclear reactions in which the reacting nuclear constituent has been identified have fixed complement, though this may not hold true in the future Only the reaction with purified DNA has given rise to precipitation (the nucleoprotein with which the LE cell factor reacts is solid at physiological salt concentrations and therefore can not enter into a precipitin reaction). Passive cutaneous anaphylactic reactions have occurred only with the LE cell factor and the factor which precipitates with DNA Thus, precipitation and passive cutaneous anaphylaxis are much less useful than complement-fixation in evaluating the types of antinuclear factors present in an individual serum The third method consists of coating inert particles, such as red blood cells or latex, with the antigen in question and observing agglutination when these coated particles are exposed to antibody These

methods are useful because they permit easy titering of individual reactivities. However, there are difficulties in attaining a reproducible coating of particles with specific nuclear constituents. The fourth method consists of observing the morphologic changes in cells induced by the antinuclear antibody. This method appears to be applicable only to the LE cell factor.

The available evidence strongly suggests that the antinuclear factors are antibodies. The reactions into which the factors enter are immunological reactions. The isolated serum factors possess both the physical and immunological characteristics of typical human antibody globulin. The serum factors demonstrate specificity for constituents of the nucleus, which would be expected of antibodies.

The most serious deficiency in the evidence that these factors are antibodies lies in the difficulty which has been encountered in obtaining analogous factors in experimental animals. In some experiments (32), immunization of rabbits with various nuclear constituents, including their own leukocyte nuclei, led to the appearance of serum factors which fixed complement only with a protein of the nucleus which is not histone. None of the experimental sera reacted with nucleic acid components of the nucleus or induced LE cell formation. However, other workers have found evidence of antibody formation to purified DNA in certain experimental animals under certain conditions (32a, 32b, 33). Miescher has published evidence of very weak ability to form cells with some resemblance to LE cells in the blood of guinea pigs and rabbits immunized with nucleoprotein (34). More recently, he has subjected rabbits and guinea pigs to intensive immunization with DNA and has obtained some sera which react with DNA by complement-fixation, conglutination, and passive cutaneous anaphylaxis (35). The identity of these serum reactions with those found in SLE serum remains to be established. Nevertheless, it appears that under circumstances of intense immunization some antibodies to nucleic acid constituents do arise in experimental animals. It is possible that the difficulty in obtaining experimental analogues of the LE serum factors lies in the fact that the animals do not have the serious derangement of immune response which appears to be present in the patients with SLE.

Formation of LE cells.—The existence of a group of antinuclear antibodies suggests the possibility that more than one of these factors is capable of inducing the formation of LE cells. This possibility cannot be excluded, but the weight of evidence is against it. There is one report that the LE cell factor reacts with an intranuclear protein alone, but the evidence presented in this report does not exclude participation of DNA in the reaction (36). On the other hand, the serum factor which reacts with the buffer extract of nuclei cannot induce LE cell formation, and there is extensive histochemical and immunological evidence which demonstrates that DNA is essential for the reaction of the LE cell factor (7, 9, 10, 13,

26). The available data therefore make it very unlikely that formation of LE cells can be attributed to a serum factor which does not react with DNA.

Seligman has suggested that the LE cell factor requires only DNA for reaction and that the factor which precipitates with purified DNA is the LE cell factor (26). The evidence for this suggestion lies in the recovery of a γ-globulin with weak ability to induce LE cell formation from the equivalence point precipitates obtained by mixing certain SLE sera and isolated DNA. Considerable evidence also exists against this hypothesis The majority of sera which cause LE cell formation do not precipitate with DNA. When the equivalence point precipitate is removed from mixtures of SLE serum and pure DNA, the bulk of LE cell activity remains in the serum. Gamma globulin recovered from equivalence point precipitates has been capable of LE cell formation only in a few instances, and the number of cells formed is small compared to the reactivity of the original serum Absorption of SLE sera with large amounts of DNA has not led to the removal of the LE cell factor. Finally, removal of histone from nucleoprotein, or substitution of protamine for histone, abolishes the reactivity of the nucleoprotein with the LE cell factor.

Thus, the evidence obtained from study of the reaction of the LE cell factor and of the reactions of the other antinuclear factors indicates that the factor which induces formation of LE cells requires both major components of nucleoprotein, DNA and histone, for reaction. This double requirement distinguishes it from other antinuclear factors which react with single nuclear constituents including DNA or histone separately. The appearance of weak ability to induce LE cell formation in the γ-globulin recovered from equivalence point precipitates of SLE serum with purified DNA, cannot be doubted However, this appears to be a minor portion of the total capacity of any individual serum to induce LE cell formation. An explanation for this reaction might lie in a weak cross reaction between the LE cell factor and DNA. An antibody requiring two components of nucleoprotein for complete reaction might be expected to react weakly with one of these components alone It is possible that different LE cell factors possess different relative affinities for DNA and histone and that in certain sera the affinity for DNA is stronger than in others Another possibility which cannot be excluded is that the factor which reacts with purified DNA possesses, on certain occasions, a weak ability to induce the morphologic changes of LE cell formation In any event, two separate serum factors arise in SLE which react with DNA. One reacts with DNA alone and possesses little if any ability to induce LE cell formation. The other reacts with the DNA and the histone of nucleoprotein and is responsible for formation of LE cells.

The precise role of histone in the reaction with the LE cell factor is

uncertain. The fact that recovery of the LE cell factor from a complex with nucleoprotein requires removal of the DNA with DNase and then elution from the histone-rich residue suggests that the factor actually binds to histone. However, histone may simply function to hold the DNA in proper configuration for binding and not participate directly in the bond. Exact information about the nature of the bond would be of considerable interest. Combination of the LE cell factor with both DNA and histone might mean that the antibody has at least two combining sites directed against two different antigenic sites. Such bispecific antibodies, while suspected in antisera to hapten-protein antigens, have not been proved to exist. It is more likely that the LE cell factor combines with the nucleoprotein at points of juncture between DNA and histone.

In addition to the LE cell factor, Aisenberg (36) has shown that another serum substance is necessary for the phagocytosis step in LE cell formation. This substance resembles complement in certain ways and is present in normal serum. One instance has been reported in which the addition of normal serum to SLE serum was necessary for LE cells to appear (36a). The precise relation of this "phagocytosis-promoting" factor to complement is uncertain. Complement, though fixed during the reaction between antinuclear factors and nuclear constituents, is not necessary for these reactions. Antinuclear factors can be completely absorbed from serum by nuclei or nucleoprotein in the absence of complement.

The present data therefore suggest that the formation of LE cells is the result of an autoantibody to deoxyribonucleohistone acting upon the cell nucleus Antibodies to other nuclear constituents arise but appear incapable of initiating LE cell formation; they may contribute to the final morphological appearance of the altered nucleus. An additional substance present in normal as well as SLE sera is necessary for phagocytosis of the altered nucleus.

Anticytoplasmic and other abnormal immune reactions.—SLE serum is also capable of reacting by fixation of complement with substances in cell cytoplasm (19, 37, 38). The reactive cytoplasmic materials have not yet been identified, and the task is made difficult by the fact that most methods of separation of nuclei and cytoplasm will contaminate the cytoplasm with the buffer-extractable nuclear antigen. Nevertheless, evidence has been obtained that mitochondria (19, 38) and also a non-ribonucleic acid constituent of microsomes are reactive (38) In view of the frequently positive Wassermann reaction in SLE, the possibility has arisen that the Wassermann antigen is often the cytoplasmic antigen. However, this has not been true for many SLE sera (38).

The anticytoplasmic reactions have been shown only by complement-fixation. Precipitin reactions and passive cutaneous anaphylaxis have not occurred in the reactions thus far studied. The anticytoplasmic factors mi-

grate on electrophoresis and sediment on centrifugation with the γ-globulins. Most have had a sedimentation rate of 7S while some have had a rate of 19S. However, none has been isolated. Thus, the proof that the anticytoplasmic factors are antibodies is less complete than that for the antinuclear factors

The antinuclear antibodies and the anticytoplasmic factors add to the large numl [illegible] E. Antibodies to red blood [illegible] telet agglutinins (41), have [illegible] sent which prolongs the clotting time, apparently by interfering with thromboplastin (42). Another γ-globulin often gives rise to false positive Wassermann reaction (39). The glomerular lesions in the kidney (27, 28, 43) and the periarteriolar fibrosis (28) in the spleen are sites of deposition of γ-globulin These latter antibodies demonstrate cell or organ specificity and thus differ from the antinuclear and anticytoplasmic factors which do not

All previously mentioned reactions have been autoimmune reactions of an immediate type An unusual skin reaction to autologous tissues also exists which may be a delayed hypersensitivity of an autoimmune type (44). When patients with SLE are injected intradermally with homogenates of their own leukocytes, a lesion of erythema and induration appears at the injection site, arising about 15 hr. after injection and lasting up to 48 hr. This skin reactivity is independent of the existence of circulating antinuclear or anticytoplasmic factors and is also independent of the state of disease activity. The time sequence of the reaction suggests a mixture of Arthus and delayed reactions Considerable study remains to be done before the nature of the reaction is known

In addition to the above reactions all of which involve the patient's own tissues, there is evidence of an increased responsiveness to foreign antigens Thus, patients with SLE are known to develop frequent transfusion reactions and are perhaps the most prolific producers of antibodies to rare blood group substances (45, 46) There is also some evidence that they form antibodies to antibiotics more frequently than do other patients (47). However, the reactivity with foreign antigens has yet to be established in control studies using standard doses of known antigens.

The appearance of the LE cells has been reported in diseases other than SLE With the probable exception of certain unusual types of cirrhosis, sometimes called "lupoid" hepatitis (48 to 51), the majority of these diseases have been instances of hypersensitivity or diseases which fall into the rheumatic diseases category Another probable exception is the "hydralazine syndrome" which remains unexplained Antinuclear antibodies have been demonstrated by fluorescent antibody and complement-fixation techniques in scleroderma, dermatomyositis, rheumatoid arthritis, and Sjogren's syndrome (15, 30, 53). They have also been found in one case of biliary

cirrhosis. However, the antinuclear antibodies are present in the great majority of patients with systemic lupus erythematosus but only in a minority of those studied with the other diseases. Reactions with cytoplasmic constituents have been more frequent than the antinuclear reactions, occurring in cases of SLE, macroglobulinemia, biliary cirrhosis, cirrhosis of unknown origin, and Sjogren's syndrome (37, 38). The data on incidence of the reactions must be considered provisional because detailed surveys of their appearance in many diseases have not been undertaken.

Titers of the antinuclear and anticytoplasmic factors and of most of the other abnormal antibodies in SLE are highest during disease activity and disappear or diminish during spontaneous and therapeutic remissions.

Origin of the unusual antibodies.—The mechanism responsible for the appearance of the large number of abnormal immunological reactions is not understood. The profusion of unusual reactions makes it probable that the basic abnormality lies in the immunological system. This system appears to be hyperactive and to have lost its capacity to "recognize" autologous tissues. Thus, antibodies are synthesized to many substances, including autologous cell constituents, to which antibodies do not arise normally. The alternative hypothesis, namely, that autologous cell constituents themselves are altered and made antigenic, appears less probable but is not excluded. The large number of cell constituents against which antibodies appear would make it unlikely that a spontaneous and nearly simultaneous change occurs in these constituents giving them new antigenic capacities. However, the possibility is not excluded that a foreign agent, such as a virus, infects and alters the cell constituents, giving rise to a change in their antigenicity. No evidence for this exists in human disease, but such a change in antigenicity does occur in certain bacteria infected with bacteriophage (54).

Pathogenic significance of the abnormal reactions.—The pathogenic significance of the abnormal immunologic reactions is not known. It is likely that certain of the abnormal antibodies such as those to red blood cells or platelets can give rise to the hemolytic anemia or thrombocytopenic purpura which occasionally occur in SLE However, there is evidence that many of the other antibodies, in particular the antinuclear antibodies, cannot enter a viable cell *in vivo*. Thus, LE cells are found in the circulation only under extraordinary conditions of anoxia or circulatory stasis Though the LE cell factor and presumably other antinuclear antibodies cross the placenta and enter the circulation of the fetus, no ill effects have been reported in children whose blood contained such antibodies for weeks after parturition (55) Examination by the fluorescent antibody technique of the tissues of patients dying with SLE has failed to demonstrate localization of γ-globulin on cell nuclei despite the antemortem presence of antinuclear antibodies in the circulation (27, 28). Finally, preliminary

evidence suggests that the antinuclear antibodies are incapable of interfering with the growth of normal monkey kidney cells or Hela cells in tissue culture (56). Thus, there is no direct evidence of a harmful effect of the circulating antibodies *in vivo*.

However, this possibility cannot yet be excluded. The hematoxylin bodies which characteristically occur in SLE appear to be cell nuclei combined with γ-globulin. The origin of these bodies is unknown. If they result from the penetration of normal cells by antinuclear antibodies, then they will stand as evidence of an *in vivo* pathogenic effect of the antibodies. On the other hand, if they result from a combination of circulating antinuclear antibodies with nuclear materials of cells dying or disintegrating in a normal way, they may not comprise evidence for such a pathogenic effect.

The weight of current evidence supports the view that the antinuclear antibodies and anticytoplasmic factors are byproducts of an abnormal immunological system, rather than decisive pathogenic agents themselves. However, the abnormal immunological system may in some other way play a role in pathogenesis.

Mackay and his associates have reported that sera from certain patients with SLE, which were capable of complement-fixation reactions with heterologous tissue antigens, could not react with similar antigens prepared from autologous spleen or muscle (57, 58) They postulate the appearance of a protective mechanism which guards the patient's tissues from potentially harmful serum antibodies. This intriguing suggestion, if true, would have considerable bearing on the pathogenic activity of the abnormal antibodies. However, such a failure to react with autologous tissues clearly does not apply to LE cell formation, which characteristically involves the patient's cells, and has not been confirmed with other reactions. Sera from more than thirty patients with SLE have been examined for complement-fixation reactions against homogenates of their own leukocytes (44). All which fixed complement against heterologous tissue antigens also did so against autologous antigens. The antigens in the latter studies contained both nuclear and cytoplasmic materials. The antigens in Mackay's studies appear to have contained at least cytoplasm and the buffer extractable nuclear antigen The postulated protective mechanism also probably does not account for the absence of antinuclear or anticytoplasmic activity in some SLE sera, for these sera have usually been studied with heterologous antigens Nevertheless, the suggestion is of sufficient theoretical interest to merit careful evaluation.

Another observation pertinent to the problem of pathogenesis is the finding of low serum complement levels in SLE (59, 60). The mechanism of this depression is unknown. It is possible that the depression is a result of antigen-antibody reactions *in vivo* which are consuming complement and therefore constitutes evidence that such reactions occur. However, it

may reflect abnormalities in the production of complement or the presence of inhibitors.

Summary—In larger numbers than in any other disease, abnormal autoimmune reactions have been demonstrated in SLE. The reactions involve many different constituents of cell nuclei and cytoplasm; an antibody which reacts with nuclear deoxyribonucleohistone is responsible for LE cell formation The mechanism whereby the abnormal antibodies arise is unknown, and present evidence is insufficient to incriminate these antibodies in the production of the major pathological lesions of SLE.

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NEOPLASTIC DISEASE: HORMONE-PRODUCING OR HORMONE-DEPENDENT TUMORS[1]

By O H. Pearson, M.D, A. G. Pazianos, M.D, and
J. M. Dominguez, M.D.
Memorial Center for Cancer and Allied Diseases, New York, New York

This review is an attempt to describe certain developments in this field within the past year or so which appear to have particular significance to the authors, and is not intended to be an encyclopedic coverage of the literature. Only occasional references will be made to animal studies which seem to be pertinent to the problem of tumors in man.

Hormone-Dependent Tumors

The concept of hormone-dependence of cancer began with the observations made by Beatson (1) that removal of the ovaries in premenopausal women with breast cancer could produce temporary regression of the cancer in some instances, and by Huggins & Hodges (2) that orchiectomy would induce remissions in men with prostatic cancer These observations led to extended studies of the role of hormones in the origin and control of abnormal and neoplastic growth, both in man and in animals (3, 4).

The relationship of endocrine factors to the initiation and subsequent growth of experimental neoplasms in animals has been under extensive study during the past decade. The evidence for a role of hormones in tumorigenesis in experimental animals is impressive. Neoplasms of the anterior pituitary, adrenal cortex, ovary, testis, mammary gland, uterus and vagina, lymphoid organs, liver, prostate gland, and the cutaneous tissues have been induced by alterations in the endocrine environment (5). The exact relationship of these observations, however, to the development of human cancr is not clear. There is no clear-cut evidence that hormones are carcinogenic in man Nevertheless, the experimental neoplasms present convenient models for study, and there may be no fundamental biological dissimilarity between animal and human neoplasms.

One of the most interesting developments in the study of animal neoplasms is the concept of "hormone dependency." The same endocrine alterations which are necessary for the induction of tumors have been found to be at least contributory and, in some cases, essential for the continued growth of the neoplasm An example of this type of neoplasm is the thyrotropic pituitary neoplasm produced by Furth (6) in mice after thyroidectomy. Pituitary tumors induced by deprivation of thyroid hormone are initially transplantable only to athyroid recipients. These are

[1] The survey of the literature pertaining to this review was concluded in July, 1959

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of 70 patients Of this group, 37.4 per cent had objective remissions lasting at least 6 months and averaging 12 4 months The average survival after adrenalectomy for the remission group was 21.4 months, as compared to 6 3 months for the failures Other investigators (13 to 16) have obtained similar results. The adrenal cortex is known to be a source of endogenous estrogen production (17), and there is presumptive evidence that adrenalectomy induces improvement in patients with metastatic breast cancer by withdrawal of estrogens. Thus, premenopausal patients with breast cancer who fail to benefit from oophorectomy, rarely, if ever, obtain benefit from adrenalectomy; whereas patients who obtain remission from oophorectomy may obtain further improvement from adrenalectomy (11)

Luft, *et al.* (18) have presented incomplete end results of hypophysectomy in patients with metastatic breast cancer. Results were evaluated in 47 of a total of 59 women Objective remissions were induced in 57 per cent of the patients for a mean duration of about 17 months. The mean survival time in the remission group was 20 8 plus months, with 8 of 27 patients still living, as compared to 8 3 plus months in the failure group with 1 of 20 still alive. Pearson & Ray (12, 19) have presented end results of hypophysectomy in 89 patients with metastatic breast cancer. Forty-two per cent of their patients had objective remissions lasting at least 6 months and averaging 16 5 plus months, with 3 of 37 patients still in remission. The mean survival time in the remission group was 24.5 plus months, with 8 of 37 patients still living, as compared to a mean survival time of 5 6 months in the failure group. Atkins (20) has compared the results of adrenalectomy and hypophysectomy in patients with metastatic breast cancer and concluded that "in hypophysectomy we have a therapeutic measure which is almost certainly as good as, and may be better than, adrenalectomy with oophorectomy." Pearson & Ray (19) have made a similar comparison They found that the incidence of remission was not significantly different with the two procedures. The average remission was 4 plus months longer in the hypophysectomy series, but this difference was also not statistically significant. In one setting, hypophysectomy appeared to be superior to adrenalectomy, namely, in the response of patients who had previously obtained remissions from oophorectomy. In this series, 87 per cent of such patients obtained further benefit from hypophysectomy, whereas only 50 per cent were improved by adrenalectomy. This difference was statistically significant at the 5 per cent level. Kennedy, *et al.* (21) have also observed a high incidence of remissions in this type of patient.

Pearson & Ray (12) have administered human growth hormone and estrogens to patients with breast cancer who appeared to be improving after hypophysectomy Estrogen administration to five patients after hypophysectomy failed to induce exacerbation of tumor growth, suggesting that a factor from, or mediated by, the pituitary is necessary for estrogen stimulation of tumor growth Human growth hormone administered to five patients after hypophysectomy appeared to induce stimulation of tumor

"conditional" or "dependent" *neoplasms. In the thyroidectomized host, the* growth of the tumor is progressive, but if thyroid hormone is supplied, the tumors regress and disappear. During the course of transplantation through new hosts, but not in the original host, the neoplasms acquire the capacity to grow in hosts with functioning thyroid glands and are then "autonomous" In the study of models of this type, Furth (7) postulates a sequence of changes from a normal cell to hyperplasia, to dependent tumor, to autonomous tumor still responsive to hormones, and finally to full autonomy. Both autonomous and dependent *tumors can metastasize. Dependent tumors can* be arrested by restoring to normal the specific regulatory mechanism which was disturbed.

In man, there is a high incidence of cancer involving organs which are under direct endocrine regulation; namely, breast, uterus, ovaries, and prostate. Although the relation of hormones to the origin of these cancers is unknown, the role of hormones in their subsequent growth is of considerable theoretical and practical importance.

Breast cancer.—In the past few years considerable attention has been given to the removal of endocrine glands in patients with metastatic breast cancer in the attempt to obtain remissions There now seems to be general *agreement that oophorectomy in the premenopausal patient with metastatic* breast cancer is a more effective palliative procedure than the administration of hormones, such as testosterone. Evidence has been presented which suggests that the tumor regression observed following oophorectomy is attributable to withdrawal of the major endogenous source of estrogens (8) Treves & Finkbeiner (9) have reported the results of oophorectomy in a series of 143 premenopausal patients with metastatic breast cancer Forty-four per cent of these patients obtained objective remissions of their disease for an average period of 14 plus months and a median period of 9 months. In the postmenopausal patient, oophorectomy will occasionally produce a regression of the cancer, but the incidence of improvement is so low *that this modality of therapy alone is not recommended. Although* the ovaries may continue to produce some estrogens after the menopause, their contribution to the total estrogen production may not be sufficient to produce a significant alteration in estrogen levels following *ablation of* these glands.

It has now been amply demonstrated that adrenalectomy will induce regression of metastatic breast cancer in some patients who have previously been castrated. Similarly, combined ablation of ovaries and adrenal glands in postmenopausal patients will induce objective improvement comparable to the results obtained from oophorectomy alone in premenopausal patients Dao & Huggins (10) have presented the final end results in their first 52 *patients. Forty per cent* of their patients obtained objective remissions The average survival after adrenalectomy for the remission group was 27 8 months, as compared to an average of 4 9 months for the failure group. *Lipsett, et al* (11, 12) *have presented similar end results in a group*

tomy, whereas in others hormonal alterations failed to influence the growth of the cancers. It is hoped that these induced tumors may serve as useful models for laboratory studies.

Prostatic cancer.—Orchiectomy and estrogens produce worthwhile temporary remissions in a high percentage of patients with prostatic cancer (33). Attempts to extend this palliation by adrenalectomy have, for the most part, been disappointing (34, 35). Although significant remissions have been observed following adrenalectomy, the incidence and duration of the improvement have not been sufficient to recommend its routine use. Hypophysectomy is also being explored as a possible means of extending palliation in these patients (36). Preliminary observations indicate that worthwhile benefit can be obtained in some patients, but further observations are needed before its usefulness can be assessed. Brendler & Winkler (37) have demonstrated symptomatic improvement in cases of disseminated prostate cancer, but no objective regression of lesions following the administration of norethandrolone (19-nortestosterone, 17α-ethyl). In contrast to the usual effect of testosterone, the administration of norethandrolone was consistently followed by significant reductions in urinary 17-ketosteroids. Their work suggests that androgenic 17-ketosteroid values do not constitute an index of prostatic cancer activity, as suggested by other investigators (38, 39).

Endometrial cancer.—Baker (40) has recently reported that he has obtained temporary objective regression of metastatic endometrial carcinoma in 5 of 15 patients with the use of progesterone. Remissions have been sustained for periods from 3 months to 4 years. Progesterone was administered in doses up to 1000 mg. per week. This observation suggests that the growth of human endometrial cancer may also be at least partially dependent upon the endocrine environment.

Hormone-Producing Tumors

There have been extensive [illegible]

of neoplastic cells. Cancer of endocrine organs may or may not retain the capacity to produce hormones, and the capacity of neoplasms to produce hormones is not necessarily related to their growth rate or invasiveness.

Steroid hormone excretion patterns have been studied in patients with adrenal cortical carcinoma (42, 43). Although marked quantitative alterations in the urinary excretion of hormone metabolites have been found, no qualitative change has been found which is characteristic of malignant cells Hormone production by adrenal cancer may or may not be influenced by administration of corticotrophin. Liddle and colleagues (44) have reported results of a suppression test in patients with Cushing's syndrome which appears to be useful in distinguishing between tumor and hyperplasia of the adrenal cortex. Δ',9αFluorohydrocortisone in a dose of 8 mg

growth in two patients. However, the latter two patients failed to obtain significant remissions from hypophysectomy, whereas the patients in whom growth hormone produced no untoward effects did obtain objective remissions. In two patients, combined administration of estrogens and growth hormone also failed to induce reactivation of tumor growth Thus, the mechanism by which hypophysectomy induces remissions in patients with breast cancer remains obscure.

Lemon has reported results of therapy with cortisone (22) and prednisone (23) in combination with oophorectomy and thyroid in patients with metastatic breast cancer. *He observed objective "palliation" in 62 per cent* of the cortisone-treated patients and in 48 per cent of the prednisone-treated cases. The average remission time was 7.7 plus months and the average survival time of the remissions was 9.3 plus months in the cortisone-treated series. The mean duration of remission was 9.7 months in the prednisone-treated series. Lemon believes that oophorectomy combined with cortical steroid therapy rivals the results of adrenalectomy or hypophysectomy. Cortisone, or its analogues, is often useful in treating critically ill patients with breast cancer who have hypercalcemia (24) or intracranial metastases (25), but the remissions are usually of short duration.

The use of androgens and estrogens in the palliative treatment of breast cancer is well established. It is also apparent that both of these steroid hormones can produce acceleration of tumor growth in some cases (8, 26). Preliminary studies of the effects of androgens and estrogens after hypophysectomy suggest that these steroids lack both their stimulatory and inhibitory effects on the growth of mammary cancer in this setting (12). This would suggest that the action of these hormones in inhibiting tumor growth may be indirect, involving the endocrine glands. Analogues of the sex steroids are being studied in the hope of finding some that may have greater antitumor effects or less side effects. 19-Nortestosterone (27) and 9α-bromo-11-ketoprogesterone (28) have been found to be less effective than testosterone. Kennedy (29) has reported objective improvement in 37.5 per cent of 48 patients with breast cancer with the use of 17-methyl-9αfluorotestosterone. In doses of 20 to 40 mg. by mouth daily, the virilizing effects seemed to be somewhat less than with testosterone.

Attempts to determine the hormone responsiveness of mammary cancer in women prior to therapy have yielded mostly negative results Hollander *et al.* (30) have studied an estrogen-sensitive transhydrogenase in breast cancer tissue and have found this enzyme to be present in 42 per cent of the cancers studied. Preliminary studies suggest that the presence of this enzyme may be indicative of hormone responsiveness *in vivo.*

Attempts to obtain laboratory animals with mammary cancers which might serve as models for the human disease have, for the most part, been unrewarding. Huggins *et al* (31, 32) have induced mammary carcinomas in the rat by the oral administration of 3-methylcholanthrene. Many of the induced carcinomas regressed profoundly after ovariectomy or hypophysec-

tumor by x-ray examination prior to adrenalectomy. Objective evidence of a pituitary tumor appeared two to eight years after adrenalectomy for hyperplasia of the adrenal glands. These patients developed deep pigmentation of the skin, and had elevated plasma ACTH levels which were more difficult to suppress with intravenous cortisol than the elevated ACTH levels in patients with Addison's disease. Histology of the pituitary in one patient resembled most closely a chromophobe adenoma Remissions have been induced in patients with Cushing's syndrome by partial or total hypophysectomy (58 to 63). This suggests that the primary disturbance is in the pituitary, at least in some patients with Cushing's syndrome Hamwi (64) has observed a patient with Cushing's syndrome caused by adrenal hyperplasia in whom total hypophysectomy failed to alleviate the adrenal hyperfunction. This suggests that in some instances the adrenal cortex may produce hydrocortisone in the apparent absence of ACTH.

Brief mention should be made of the more unusual syndromes associated with hormone-producing tumors of the endocrine glands. Primary hyperparathyroidism is commonly caused by one or more parathyroid adenomas, but rarely are four adenomas of the parathyroids found, as described by Moldover *et al* (65). Functioning parathyroid carcinomata are an even rarer entity. Cook (66) points out that there are only 22 cases in the world literature to date of hyperparathyroidism secondary to carcinoma of the parathyroid gland. The effect of parathormone on calcium and phosphorus metabolism is well known In addition, it may have a direct effect on reducing magnesium stores in the body, as recently reported by Agna & Goldsmith (67) In considering the diagnosis, the familial incidence of hyperparathyroidism should not be overlooked (68)

Until recent years the diagnosis of pheochromocytoma was often difficult to establish. With the advent of a method for measuring catechol amines in body tissues and fluids (69), the diagnosis of this tumor of the adrenal medulla has been greatly simplified Scattered reports in the literature of familial pheochromocytoma (70 to 74) suggest a possible genetic mechanism The frequent association of this tumor with neurofibromatosis is further evidence for a genetic implication (75 to 77).

Among the less common tumors of the adrenal cortex are adenomas which secrete primarily aldosterone. Since the syndrome of primary aldosteronism was first described by Conn & Louis (78) and by Conn (79), a great deal has been written about its clinical manifestations and its pathologic physiology This subject has been reviewed recently by August (80). It has been repeatedly stated that edema does not occur in this syndrome. However, a well-documented case in which edema was a primary feature has been described (81).

More common endocrine tumors are those arising from pancreatic islet cells and producing the syndrome of hyperinsulinism. The diagnosis may be overlooked for a long period of time since these patients are often thought to be psychotic, as pointed out by Marshall (82), Doorly & Martin (83), and

daily is administered for 48 hr. The urinary excretion of 17-hydroxycorticosteroids is not suppressed in patients with cancer, whereas it is regularly suppressed in patients with hyperplasia of the adrenal cortex.

Hormone production by malignant neoplasms may be a useful index in the exploration of chemotherapeutic agents. Chemical agents capable of blocking hormone production may be active in malignant hormone-producing tumors without influencing the growth of the tumor. Thus, amphenone (3,3-bis(*P*-aminophenyl)butanone-2) has been shown to inhibit steroidogenesis in patients with adrenal cortical carcinomas without apparent effect on the growth of the neoplasm (45 to 47). Amphenone inhibits the production of both 11-oxyketosteroids as well as the 11-deoxyketosteroids Another compound, 2-methyl-1,2 bis(3-pyridyl)-1-propanone (SU-4885), has been found to inhibit 11β-hydroxylation of steroids by the normal adrenal cortex as well as by carcinoma of the adrenal cortex (48, 49). In a patient with adrenal carcinoma, SU-4885 produced a striking decrease of all the 11-oxygenated steriods examined This was accompanied by a roughly equivalent increase in the metabolites of Reichstein's substance "S." There were changes in the patient suggestive of adrenal insufficiency but there was no evidence of inhibition of tumor growth. On the other hand, Bergenstal *et al* (50) have reported both suppression of adrenal function and regression of adrenal cancer with the use of *ortho, para'*dichlorodiphenyl dichlorethane. Thus, there is emerging a group of chemical compounds which show promise for the eventual control of adrenal hormone production as well as inhibition of neoplastic growth

Recent reports indicate that the thyroid gland under certain circumstances may continue to produce thyroid hormone in the absence of the pituitary thyrotropic hormone Werner & Stewart (51) and Fajans (52) have reported the occurrence of hyperthyroidism in patients with panhypopituitarism. Gurling *et al.* (53) have observed the development of a toxic nodular goiter in a patient who had undergone apparent total hypophysectomy Becker (54) has reported the failure of suppression of thyroid function after hypophysectomy in two patients with Grave's disease. Pearson *et*

production of thyroid hormone. One patient had a previous history of thyrotoxicosis and two appeared to have normal thyroid glands. The observations that hyperthyroidism may develop in the absence of the pituitary does not necessarily mean that hyperthyroidism is always unrelated to the pituitary. Bottani (56) has shown that serum TSH levels are sometimes normal and sometimes markedly elevated in patients with hyperthyroidism

Nelson *et al* (57, 57a) have reported the development of adrenocorticotrophic hormone secretory tumors of the pituitary gland in patients who had previously undergone bilateral adrenalectomy for the treatment of Cushing's syndrome In three patients there was no evidence of pituitary

In general, masculinizing ovarian tumors are not associated with markedly elevated 17-ketosteroid excretion. Cohen (111) pointed out that the cortisone suppression test may be useful in differentiating between masculinizing ovarian tumors and adrenal hyperplasia. Another useful differentiating test is the chromatographic separation of urinary 17-ketosteroids Keller *et al* (112) observed excessive amounts of dehydroepiandrosterone in a case of virilizing adrenal tumor, whereas this fraction is not elevated in cases of masculinization associated with gonadal tumors.

Feminization may, paradoxically, be produced by interstitial cell tumors of the testicle, when these tumors occur in adult life. Approximately twelve cases of this type have been described (113 to 115). Generally, this syndrome is characterized by bilateral gynecomastia, impotence, loss of libido, and sterility. Magendie *et al.* (116) described one case in which only gynecomastia was present. In the cases in which urinary estrogens have been studied, the values have usually been above the normal range for males. This same paradox has been demonstrated in the experimental animal by Huseby (117) and by Gardner (118), who showed that interstitial cell tumors in the mouse were capable of both androgen and estrogen production. Sertoli cell tumors of the testicle may also produce feminization (119 to 121). Although rare in the human being, this tumor, interestingly enough, appears with relative frequency in the dog (122). Only one case has appeared in the world literature of granulosa cell tumor of the testicle with high estrogen production and feminization (123) Similarly, feminization may occur in patients with testicular choriocarcinoma (124, 125).

Approximately 35 cases of feminization in the adult male have been described (126) associated with adrenocortical carcinoma. Of unusual interest is Smaith's recent report (127) of adrenal tumor presenting as isosexual precocity in a girl 5½ years of age. The level of 17-ketosteroid excretion suggested the presence of a tumor Of particular importance in this case was the absence of signs of Cushing's syndrome or of abnormal virilism, and the gross similarity clinically to true precocious puberty.

A bizarre characteristic of malignant neoplasms is the occasional occurrence of the production of hormones, or the tripping off of excessive hormone production, which is not usual to the tissue of origin. High titers of urinary chorionic gonadotropin have been found in patients with adrenal cortical carcinoma, breast carcinoma, and malignant melanoma (128, 129). An inadvertent finding that amethopterin suppressed the excretion of chorionic gonadotropin in a patient with melanoma led Li *et al* (130) to explore the use of this antimetabolite in patients with choriocarcinoma. Remarkable regressions of widespread metastatic choriorcarcinoma have been achieved with the use of amethopterin and other antimetabolites in women with this trophoblastic neoplasm (131, 131a). Li *et al.* (132) have also reported successes in the treatment of testicular choriocarcinoma with the use of chemotherapeutic agents.

Other examples of unusual hormone production associated with neo-

others On the other hand, the association of functioning islet cell tumor with diabetes mellitus is a rare entity. To date, less than ten cases have been reported. Gittler, *et al* (84) cite an interesting case in which the diabetes mellitus was ameliorated by an insulinoma. Functioning islet cell carcinomas are also uncommon. Landau (85) reported the effect of prolonged glucagon administration in a patient with a functioning islet cell carcinoma and observed a hyperglycemic response.

A great deal of confusion has existed in the literature because of the nomenclature of ovarian, testicular, and adrenal tumors producing sex hormones. Teilum (86) has attempted to clarify our understanding of certain ovarian and testicular neoplasms by classifying them according to histological similarity and embryological derivation. From an endocrinologic standpoint, it is perhaps simplest to consider these functioning tumors as producing feminizing or masculinizing syndromes.

Among the tumors which may produce masculinization are interstitial cell tumors of the testicle when they occur prior to puberty. Twenty cases have been described to date (87 to 90). In addition to masculinization, only two of these cases had gynecomastia (91, 92). With the exception of one (93), all of these tumors have been described as benign, and they are generally unilateral Staubitz *et al.* (94) described one case in which bilateral interstitial cell tumors were present. However, the eventual course of this patient and that of the case of Wilkins (95) suggests that this was, in actuality, a case of adrenogenital syndrome with congenital adrenal hyperplasia and adenomatous hyperplasia of the interstitial cells of the testicles In these cases of functioning interstitial cell tumors, the 17-ketosteroid excretion may be increased a few milligrams (96) or as much as 1000 mg. (97), the larger fractions being etiocholanolone and androsterone (98 to 100).

Masculinization associated with ovarian tumors is caused most commonly by arrhenoblastoma. Wiest *et al.* (101) have recently shown the capacity of tissue from arrhenoblastoma to metabolize progesterone *in vitro* to 17-hydroxyprogesterone, androstenedione, and at least two more substances which are not identified. During the past forty years, approximately thirty-five cases of masculinization arising from ovarian tumors bearing a close histological resemblance to adrenocortical tissue have been described. These have been referred to as "masculinovoblastoma" and "virilizing lipid cell tumors" (102 to 104). Abouab *et al* (105) have recently described a case of this type in which there was hypercalcemia. The serum calcium reverted to normal following surgery and the authors postulated the elaboration of a parathormone-like substance by the tumor. More rarely, masculinization may occur in association with hilus cell tumors of the ovary which are similar to the interstitial cell tumor of the testicle (106 to 109) One case of granulosa cell tumor associated with masculinization was described by Mackinlay (110). Pathologic examination of the tumor revealed Leydig cells in some areas.

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Other examples of unusual hormone production associated with neo-

plasms have been described. August & Hiatt (133) have reported severe hypoglycemia secondary to a non-pancreatic fibrosarcoma with insulin activity. Adrenocortical carcinoma associated with hypoglycemia of undetermined cause was described by Aszkanazy *et al.* (134). Sellman (135) reported a patient with mesothelial cell sarcoma associated with hypoglycemia and speculated that excessive glucose consumption by the tumor may have been responsible for the hypoglycemia in their patient. Warner & Southern (136) and Stanford *et al.* (137) have reported the appearance of the carcinoid syndrome in patients with metastasizing bronchial adenoma

Other endocrine abnormalities associated with neoplasms suggest that the neoplasm may incite inappropriate hyperfunction of endocrine glands Cushing's syndrome arising from adrenal hyperplasia has been found in association with a variety of malignant tumors (138 to 143). Alterations in calcium and phosphorus metabolism, usually considered characteristic of hyperparathyroidism, may be associated with a number of different types of neoplasms (144, 145). Removal of the primary neoplasm has, in some instances, resulted in the return of calcium and phosphorus to normal, as though a parathyroid adenoma had been removed (146). Schwartz *et al* (147) have reported a syndrome of renal sodium loss and hyponatremia probably resulting from an inappropriate secretion of an antidiuretic hormone in patients with neoplasms.

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NUTRITION AND NUTRITIONAL DISEASE[1]

By Norman Jolliffe, M.D and Robert S Goodhart, M D.
Bureau of Nutrition,
The City of New York Department of Health, New York, N Y.

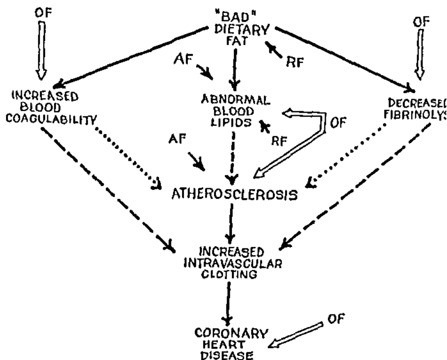
We are not here attempting a review of all recent work in nutrition. Rather, we have been deliberately selective. For the most part we have chosen to discuss work and developments not touched upon in this section during the past four years; here, again, selecting those items which we considered to have actual or potential clinical significance. A good over-all picture of recent progress in the study of nutrition and nutritional diseases can be obtained by reading the contributions on this subject which have appeared in the *Annual Review of Medicine* from 1956 to-date.

Role of Fat Quality in Pathogenesis of Coronary Heart Disease

Certain provisional schemata for the possible roles of fat in the development of coronary heart disease have been postulated Ahrens *et al.* (1) have suggested that the pathway proceeds from "bad" dietary fat to abnormal serum lipids to atherosclerosis to coronary disease O'Brien (2) indicated that the schema should be modified to include blood clotting and fibrinolysis, with less emphasis on the role of atherogenesis Jolliffe (3) devised a schema which combined the ideas of the Ahrens' group (1) and of O'Brien (2) with the role of other dietary and non-dietary factors.

Jolliffe's (3) schema includes three possible mechanisms (Fig. 1) by which "bad" dietary fats may lead to coronary heart disease. The direct line through the center is essentially the Ahrens' schema The line between "bad" dietary fat and abnormal blood lipids is solid to indicate its firmness The accentuating and retarding factors recognize the modifications introduced by gonadotropic and thyroid hormones, conditional factors, and such disease factors as diabetes, nephrosis, and idiopathic hyperlipemias. The line between abnormal blood lipids and atherosclerosis is broken since this is not yet proved to the complete satisfaction of many people One double-shafted arrow at "abnormal blood lipids" and "atherosclerosis" is to include Ahren's (1) caution that both hypercholesterolemia and atherosclerosis may be genetically determined, and that the two manifestations need not be causally related The line between atherosclerosis and increased intravascular clotting is solid, for slowing of the blood stream by severe narrowing of the arterial lumen is known to promote intravascular clotting. But, "bad" dietary fats do not necessarily have to produce coronary heart disease through the atherogenic pathway The increased blood coagulability and decreased fibrinolytic activity caused by certain dietary fats seems

[1] The survey of the literature pertaining to this review was concluded in August, 1959



Revision of possible pathways from ingested fat to coronary disease. ——> Highly probable to proved; - - - -> possible to probable; · · · ·> theoretical. AF ——> accentuating factors, e g, genetics, hormones, diabetes, nephrosis maleness; RF ——> retarding factors, e g, genetics, hormones, femaleness; OF ═══> other factors outside schema unrelated to dietary fats

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important. These two influences, if effective *in vivo*, could only lead to increased intravascular clotting, especially when associated with a slowing of the blood stream by an atherosclerotic plaque The double arrow pointing to these two pathways recognizes factors other than dietary fats. It may well be that the factors of coagulability and fibrinolysis account for the presence or absence of clinical coronary heart disease in different persons with relatively equal amounts of coronary atherosclerosis. The double arrow pointing to "coronary heart disease" recognizes the other etiologic factors of coronary heart disease, such as aneurysms, embolizations, etc.

Effect on Human Serum Lipids of Highly Unsaturated Fats Poor in Essential Fatty Acids

Ahrens *et al* (4) prepared an oil for human consumption from the body

acids. Ahrens' group first caused a fall in the serum cholesterol, phospholipid, and triglyceride levels in 2 subjects by substituting a formula diet containing 40 per cent of the calories as corn oil for the ad libitum diet. When menhaden oil (iodine number 179) was substituted isocalorically for corn oil (iodine number 126) serum cholesterol, phospholipid, and triglyceride levels remained depressed, falling even further in one subject. They concluded that the effects on serum lipid levels in these two patients (one with hyperlipemia, the other with hypercholesterolemia) were unrelated to the essential fatty acid and sterol contents of the fats used but supported their hypothesis that the falls in serum lipids were more related to total unsaturation than to the essential fatty acids of the dietary fat. A recent report by Keys, Anderson & Grande (5) describes the response of the serum cholesterol in 6 subjects to a mixed diet containing about 35 per cent of its total calories as fat, of which about 70 gm. were saturated fatty acids, about 67 gm mono-ene fatty acids and about 6 gm. poly-ene fatty acids. When 4 to 5 gm. of arachidonic acid in the form of arachidonic acid concentrate in capsules was added to the diet, the changes in the serum cholesterol were unimpressive. This lends no support to the idea that supplementing an ordinary diet with the most highly unsaturated of the essential fatty acids (arachidonic) will produce any important depression of the cholesterol level

PUBLIC HEALTH APPROACHES TOWARD LOWERING SERUM CHOLESTEROL LEVELS

Previous reviews in this series have amply demonstrated that substitution of mixed dietary fats with formula feedings containing large amounts of polyunsaturated fatty acids from vegetable oils or marine oils results in a fall of blood cholesterol as well as certain other blood lipid fractions It is now well recognized that formula feedings, although experimentally highly successful, do not constitute a way of life, a practical eating pattern for the population at large or even for the hypercholesterolemic subject. Consequently, the first cautious approaches have been made during the past year to practical diet patterns, made up of foods available in every community and suitable for a free-living, ambulatory subject

Jolliffe, Rinzler & Archer (6) placed 79 normal weight men, aged 50 to 59, on a diet of 2000 to 2700 calories with fat supplying 30 to 33 per cent of the calories. Saturated fatty acids supplied 7 to 8 per cent of the calories, monounsaturated fatty acids (principally oleic acid) supplied 8 to 9 per cent and polyunsaturated fatty acids supplied 9 to 11 per cent. After six months on the diet, the group's cholesterol average had fallen from 251 to 222 mg per cent. By tertiles, the levels fell from initial averages of 298, 250, and 204 mg per cent to 252, 225, and 189 mg. per cent, respectively. The decreases in each tertile were statistically significant beyond the 1 per cent level.

Brown & Page (7) found that a vegetable oil diet very low in saturated fats but containing about 3 ounces of vegetable oil significantly lowered the blood cholesterol level in 16 subjects, 15 of whom were hyperlipemic. All of their subjects were trained by eating for a time in an experimental diet kitchen

Meltzer, Bockman & Berryman (8) studied the effect of a pharmaceutical emulsion of safflower oil plus sitosterol plus pyridoxine plus vitamin E on the blood cholesterol level of 28 hypercholesterolemic-myocardial infarction patients aged 36 to 53. This emulsion was administered for six months as a dietary supplement, with instructions to the patients to maintain their previous fat intake. The over-all change after six months was a significant decline in serum cholesterol for all 10 patients consuming less than 50 gm. dietary fat daily; for 7 of 8 patients consuming 50 to 100 gm. dietary fat daily; and for 7 of 10 patients consuming 100 to 150 gm. of dietary fat daily

Boyer *et al* (9) found, in a nine month's study of 300 in-patients in a state institution, that a regimen which substituted a margarine containing 20 per cent hydrogenated coconut oil and 80 per cent unaltered corn oil for other table and kitchen fats as the principal dietary change was effective in achieving and maintaining a decrease in blood cholesterol levels.

This study indicates that much could be done in changing the level of blood cholesterol in the American population if industry would cooperate in providing cooking and table fats of a suitable fat quality.

Vitamin A

In continuation of their studies of the function of vitamin A in metabolism, Wolf *et al* (10) found the depression of glyconeogenesis observed in the intact vitamin A-deficient rat to be reversed by cortisone but not by adrenocorticotropic hormone treatment It was demonstrated histologically that there is a disturbance in those adrenal cortex cells producing glucocorticoid hormones before glyconeogenesis depression occurs Wolf *et al.* concluded that vitamin A deficiency leads first to a degeneration of cells in the adrenal cortex and only secondarily to a depression in glyconeogenesis through impaired glucocorticoid hormone production Varandani, Wolf & Johnson (11) found a marked decrease in the formation of mucopolysaccharide by excised colons and colon homogenates, from vitamin A-deficient rats This could be reversed by the *in vitro* addition of vitamin A and was found to be specific for vitamin A Rice & Bo (12) have reported that cortisone has no demonstrable effect on the conversion of beta-carotene to vitamin A

Of greatest importance in respect to the visual system is the difference in distribution of vitamin A isomers in the eye tissues from that in the rest of the body Krinsky (13) has shown that a large percentage of the vitamin A present in pigmented layers of cattle eyes and retina is the neo-b

isomer, the precursor of rhodopsin This isomer has not been found in either cattle plasma or liver. This fact, coupled with the retention by the eye of vitamin A during depletion, points to a mechanism for isolating the vitamin A metabolism of the eye from the rest of the body.

According to Dowling & Wald (14), vitamin A acid is able to fulfill all somatic needs for vitamin A in the rat, except that the rat seems unable to reduce it to the alcohol, or to the aldehyde, retinene. Rats maintained on vitamin A acid, though otherwise in good condition, become highly night-blind Also, animals fed vitamin A acid do not store detectable amounts of either the acid or the alcohol For this reason such animals, on withdrawal of the vitamin A acid, begin to lose weight within a week.

The teratogenic effects of excessive amounts of vitamin A in the rat can be largely prevented by the simultaneous administration of a combination of thiamine, niacinamide, riboflavin, and pyridoxine, according to Millen & Woollam (15). In a group of rats that received 40,000 I U. of vitamin A acetate daily by gastric intubation without additional B vitamins, 7.4 per cent of the young developed abnormalities of the brain and 31 4 per cent had cleft palates In rats receiving the same amount of vitamin A plus the parenteral B vitamins in large amounts, none of the young showed brain damage and the incidence of cleft palates was only 1.5 per cent.

Roels, Trout & Dujacquier (16), working with vitamin A-deficient boys in the Belgian Congo, found that the daily administration of 200 gm of carrots resulted in the absorption of less than 5 per cent of the carotene contained therein When 18 gm of olive oil was fed daily along with the supplement of carrots, 25 per cent of the carotene was absorbed The administration of carotene in oil resulted in the absorption of 45 per cent of the carotene Roels and his associates suggest that the addition of fats to the diet may contribute to the relief of vitamin A deficiency in this region

Horvat & Maver (17) report on the study of 80 children in the village of Krk on the island of Krk in the Northern Adriatic. The children were divided into two groups of 40 each, and each member of the experimental group received a daily supplement of 3000 I.U of vitamin A for three months At the beginning of the experiment the mean serum vitamin A level was 15 5 ± 0 68 µg per cent in the experimental group and 15.0 ± 0 64 µg per cent in the controls The incidence of goiter in the experimental subjects was 66 per cent; while in the controls it was 67 per cent None of the children showed any clinical signs of vitamin A deficiency At the end of the three-month treatment period the mean serum vitamin A in the experimental group was 22 3 ± 0 99 µg per cent and the incidence of goiter was 37 per cent The corresponding figures for the control group were 15 4 ± 0 81 µg. per cent, and 64 per cent

Horvat & Maver conclude that their investigations indicate that the deficiency of vitamin A on the island of Krk should be regarded as an

important factor in the occurrence of goiter on that island. "Moreover, this contributes to the opinion that goiter cannot always and exclusively be attributed to absolute deficiency of exogenous iodine, but should be regarded as the consequence of a complex nutritional deficiency. The amount of vitamin A in food may be an important element in the genesis of goiter and in many cases it may serve as a therapeutic factor for its decrease" (17).

In the United States, Wolf (18) has reported the case of a three-month-old allergic infant, born to a mother who was on a low fat diet and itself fed a soy bean milk without added vitamins, who developed vitamin A deficiency in which both eyes were affected with loss of vision in one.

Vitamin D

Fellers & Schwartz (19) found excessive serum vitamin D activity to be of primary importance in the severe form of idiopathic hypercalcemia of infancy. They found further that the exclusion of exogenous vitamin D had no effect on the clinical and chemical condition, and concluded that this disease should be included in the rapidly growing list of inborn errors of metabolism, or molecular diseases. In contradiction to the findings of Fellers & Schwartz (19), Thomas *et al* (20) found the sera of two infants with idiopathic hypercalcemia to contain normal quantities of antiricketic substance The mean antiricketic activity of sera of 18 normal subjects was found to be equivalent to two I.U. of vitamin D per ml, and this was not increased in the sera of patients with a variety of non-vitamin D-induced hypercalcemic states. Failure of vitamin D absorption alone did not account for the hypocalcemia noted in three patients with non-tropical sprue.

Vitamin E

The question whether vitamin E has a metabolic function other than that of an antioxidant has not been resolved. Machlin, Gordon & Meisky (21) concluded from their studies that in the chicken, as has been reported for the rat, the requirement for vitamin E is largely represented by a need for a biologically active antioxidant Similarly, Bieri & Briggs (22) found that in the presence of dietary selenium α-tocopherol has no biochemical function in the growing chick other than that of a nonspecific antioxidant; however Gitler, Sunde & Baumann (23) have pointed out that the several symptoms of vitamin E deficiency may develop independently of one another, and that substitutes for vitamin E correct only certain of these symptoms, while tocopherol itself corrects all of them Ferguson *et al* (24) have reported that vitamin E increases the hatchability of turkey eggs 12 to 18 per cent, while selenium has no vitamin E-like activity in this respect. The decline in succinate oxidation in the presence of DPN has been observed to be greater in liver mitochondria from rats deficient in vitamin E than in mitochondria from vitamin E-supplemented animals Here, again, Factor 3, in the form of sodium selenite, is without effect (25).

Gray & Loh (26) administered 100 mg. of α-tocopheryl acetate daily to healthy human subjects for ten days and obtained significant increases in total cholesterol, free cholesterol, and phospholipids in the plasma, indicating the implication of vitamin E in the metabolism of fats They also found a significant increase in the α-2 globulin fraction of the plasma proteins and a significant decrease in plasma amino acids, suggesting involvement in the metabolism of protein.

Horwitt (27) has reported on the preliminary analyses of data obtained during a five-year controlled study of human tocopherol requirements. In this study the prolonged feeding of an unsaturated lipid, which had been oxidized to remove vitamin E, was followed by duodenal ulcers in about one-third of the subjects. It was also observed that (*a*) the tocopherol levels of tissues can be related to the amounts of oxidizable lipid consumed; (*b*) the recovery of erythrocytes in their response to the peroxide hemolysis test, when 60 mg. of α-tocopheryl acetate are given daily, lags behind the more rapid return of plasma tocopherol to predepletion levels; and (*c*) the administration of 200 mg. of D-α-tocopheryl acetate produced a small but significant reticulocytosis in those experimental subjects whose hemoglobin levels were 13 0 gm. per cent or less.

Horwitt & Bailey (28) have reported observations on the brain of an infant who had been hospitalized because of an abdominal tumor and who had been fed intravenously a diet high in unsaturated fat and devoid of vitamin E for a period of 21 days preceding death. This infant's brain showed a hemorrhagic endarteritis of the cerebellum histologically similar to the changes noted in the cerebellum of vitamin E-deficient chicks which had been fed unsaturated fats

Rose & György (29) have presented experimental evidence, with the rat, supporting the view that vitamin E deficiency is implicated in the adverse effects of large doses of water-soluble vitamin K analogues in newborn infants.

Factor 3 and Selenium

Factor 3 is a water-soluble organic compound of low molecular weight containing selenium as an integral constituent. It is unrelated to vitamin E, but is very effective in preventing certain deficiencies most of which previously had been attributed to lack of vitamin E. These include liver necrosis in the rat; multiple necrotic degeneration in the mouse; exudative diathesis

of L-cystine are explainable on the basis of contamination of commercial L-cystine with traces of selenium. This is denied by Yang, Dialameh & Olson (32).

Factor 3-active selenium compounds do not substitute for vitamin E. The course of muscular dystrophy in young rabbits maintained on vitamin E-free

diets is not influenced by added sodium selenite and selenocystine [Hove, Fry & Schwarz (33)]; nor do Factor 3-active selenium compounds show any vitamin E activity by bioassay with the standard rat resorption-gestation technique [Harris, Ludwig & Schwarz (30)]. Conversely, selenium has a definite protective effect against "white muscle disease" (a myopathy in lambs and calves), a condition in which vitamin E is ineffective [Muth *et al.* (34)].

Nesheim & Scott (35) have advanced evidence that selenium is a required nutrient per se, in chicks, necessary for growth as well as for prevention of exudative diathesis They also have shown that selenium is partially effective in the prevention of muscular dystrophy in chicks. The selenium requirement for growth in the presence of vitamin E is approximately two-thirds the requirement for preventing exudative diathesis, when fed alone. Vitamin E cannot completely replace the need for selenium, according to these workers

Ascorbic Acid

L-Ascorbic acid is synthesized in rats as follows: D-glucose (or D-galactose) → D-glucuronic acid → L-gulonic acid → L-gulonolactone → L-ascorbic acid (36 to 39). All animals studied can metabolize L-gulonic acid via L-xylulose, but only man, the other primates, and the guinea pig appear to lack the ability to convert L-gulonic acid to L-ascorbic acid It has been postulated that this is the genetically determined missing step which makes necessary the inclusion of ascorbic acid in the diet for the prevention of scurvy (36); however, recently Baker, Plough & Bierman (40) fed glucuronolactone to human subjects and obtained an increase in the urinary excretion of both xylulose and ascorbic acid, suggesting that man can synthesize ascorbic acid [illegible] in man is rela-

Raiha (41), in studies on samples of maternal and cord blood, found that in all cases studied the fetal blood contained more total ascorbic acid than the maternal blood Also, when the maternal plasma total ascorbic acid exceeded the mean normal value of 0.60 mg per cent, an accumulation of total ascorbic acid was found in the amniotic fluid, whereas when the fetal plasma showed levels below the mean normal value of 1 20 mg per cent the amniotic fluid contained an even lower total ascorbic acid concentration Studies on the guinea pig showed that infusion of dehydroascorbic acid into the maternal blood brought about a clear increase of total ascorbic acid in the amniotic fluid and a marked increase of total ascorbic acid in the fetal blood, especially in the erythrocytes This increase was not found when ascorbic acid was infused *In vitro* studies with adult and fetal human blood showed that additions of dehydroascorbic acid resulted in marked increases in total ascorbic acid content of the erythrocytes When ascorbic acid was

added, nearly all of the vitamin remained in the plasma. Räiha (41) comments that the transfer of vitamin C across the placenta most probably occurs as dehydroascorbic acid, and that the high content of vitamin C in the fetal blood is caused mainly by an increase in the concentration of L-ascorbic acid on the fetal side of the placenta. This mechanism of transfer would make it possible for the fetus to maintain a high concentration of vitamin C.

Pankamaa & Räiha (42) studied the frequency of stillbirths at different times of the year, from 1924 to 1953, at the Women's Clinic of the University of Helsinki, Finland. During this period 116,790 infants weighing not less than 600 gm were born Of this number, 1801 died in utero Excluding 397 cases in which the cause of death was unknown, they found that the frequency of stillbirths showed a seasonal change of about 40 per cent, which change was inversely proportional to the seasonal fluctuation in vitamin C content of fetal brain. No seasonal variation could be demonstrated in the group of excluded cases and it was suspected that many of these deaths were caused by crime

Pye, Taylor & Fontanares (43) placed different groups of guinea pigs on diets containing 2 mg, 4 mg, 6 mg, and 8 mg of ascorbic acid The average reproduction record of the group receiving 8 mg of ascorbic acid daily "was superior in every respect to that of all the other groups." The increase in young born alive in the 8 mg group over the 2 mg group was 302 per cent.

Because of a recent increase in reported cases of infantile scurvy in Toronto and Winnipeg which he considers to be due to the decline in breast feeding, the reluctance of some mothers to give orange juice, and general confusion in the lay mind about vitamin concentrates, Medovy (44) has recommended that all evaporated milk be fortified with vitamin C. Cutforth (45), in a report of 11 cases of adult scurvy occurring in England, has emphasized that the "clinical picture of scurvy seen in this series of cases differs in several respects from the descriptions found in current textbooks of medicine. In his patients the chief symptoms were pain, lethargy, anorexia, and mental depression The chief signs were small bruises on the limbs, extravasation of blood into the tissues and anemia Typical scorbutic lesions of the gums were seen in only one case, but only four patients had any teeth

Von Schuching, Abt & Roe (46) found that although wound healing (in guinea pigs) took place at blood levels of ascorbic acid as low as 0.17 to 0 12 mg per cent, the formation of strong scar tissue was more rapid

pendent of the vitamin [Gould (47)]. Crandon *et al.* (48) found 19 of 47 patients suffering wound dehiscence to have severe ascorbic acid deficiency (plasma ascorbic acid levels below 0 2 mg per 100 ml. together with buffy-coat levels below 8 mg. per 100 gm.) They observed that surgical patients

suffering severe inflammatory processes required 300 mg of ascorbic acid daily.

Investigations continue on possible relationships between the flavonoids and ascorbic acid metabolism Douglass & Kamp (49) give a fillip to this with their finding that the addition of rutin to the diet of guinea pigs receiving less than optimal amounts of ascorbic acid results in an increased adrenal ascorbic acid content As expected from the fact that flavonols are rapidly destroyed in liver tissue but are relatively stable in adrenal homogenates, no effect of rutin on liver ascorbic acid levels was observed Anderson, Coots & Halliday (50) were unable to demonstrate any antiscorbutic activity of inositol in guinea pigs. In fact, they confirmed the finding of others that the guinea pig has no dietary requirement for inositol.

Erythorbic acid is widely used as an antioxidant in foods and Dodds, Fisher & Wang (51) point out that this can lead to confusion concerning the state of ascorbic acid nutriture as judged by blood levels While erythorbic acid is relatively inactive as an antiscorbutic substance, it can raise the blood and urine level of apparent total blood ascorbic acid

VITAMIN B_6

"Vitamin B_6 in Internal Medicine" was the subject of a 1958 review by Wayne *et al.* (52) They point out that this vitamin is a very important substance in the diet of human beings. Its chemical and biological activities suggest that it may have an important bearing on the arteriosclerosis problem and on the function of the central and peripheral nervous system, the bone marrow, the skin, and the mucous membranes. Recent work casts some doubts on the validity of Vilter's conclusion (53) that the vitamin B_6 requirement of adults is between 1 to 2 mg daily and that this can be readily met by the average American diet Harding, Plough & Friedemann (54) found the minimal daily requirements of nine, healthy, young, male adults, as determined by xanthurenic acid excretion after a test dose of tryptophan, to be between 1.93 and 2 76 mg. They also found that prolonged storage (20 months) of packaged rations (army C ration) at high temperature (100°F.) was associated with appreciable losses of vitamin B_6. Boxer, Pruss & Goodhart (55) found the pyridoxal phosphate content of the leukocytes of presumably healthy New York City school children to be significantly lower than that of the leukocytes of Cuban children of the same age. Adults had even lower levels of pyridoxal phosphate. Wachstein, Moore & Graffeo (56) have reported that the pyridoxal phosphate content of leukocytes of pregnant women is lower than that of non-pregnant women, and that it can be raised to "almost normal control levels" by oral administration of pyridoxine to pregnant women. These workers found that the growing fetus can successfully compete with the mother for vitamin B_6. From studies on rats, Wachstein & Moore (57) concluded that measurement of pyridoxal

phosphate in leukocytes is a sensitive indicator for assessment of nutritional status in regard to vitamin B_6.

Gershoff, Mayer & Kulczycki (58) studied the excretion of various metabolites by mongoloid and non-mongoloid mentally deficient patients receiving a diet "apparently adequate" in vitamin B_6 (estimated content more than 1 to 2 mg daily). The administration of pyridoxine brought about a marked reduction in the urinary excretion of oxalic acid in both groups Following pyridoxine administration, mongoloids excreted more pyridoxic acid and less vitamin B_6 than did non-mongoloids Oxaluria, as a manifestation of vitamin B_6 deficiency, has been demonstrated in rats (59, 60) and in the cat (61). In vitamin B_6 deficiency there appears to be a marked increase in the endogenous production of oxalic acid probably derived in part from glycine (60, 61).

Ranke *et al.* (62) found persons over 60 years of age to have significantly lower serum glutamic acid-oxaloacetic acid transaminase (SGOT) levels than did adults under 40 years old. Pyridoxine administration to the elderly persons brought about a significant increase in the average SGOT level. Lerner, DeCarli & Davidson (63) have reported an association between vitamin B_6 deficiency and convulsions in alcoholism. The findings in patients with "rum fits" that tryptophan loading resulted in the excretion of large amounts of xanthurenic acid and that correction of this metabolic defect occurred after the administration of pyridoxine suggest that "rum fits" are etiologically related to vitamin B_6 deficiency. Gamma radiation of raw beef destroys about 25 per cent of its vitamin B_6 (64), and cooking of meats brings about an average destruction of about 46 per cent of the vitamin B_6 (65).

Yeh & Chow (66) report that vitamin B_6-deficient rats show a failure to absorb vitamin B_{12}, a defect which cannot be corrected by administering intrinsic factor, but which can be corrected by injection of cortisone. They suggest that vitamin B_6 deficiency causes adrenal cortical dysfunction; however, Eisenstein (67) was unable to demonstrate any adrenal dysfunction in vitamin B_6-deficient animals, although the adrenals were hypertrophied Gantt, Chow & Simonson (68) report marked early impairment of conditional reflexes in both vitamin B_6-deficient rats and dogs.

Coursin & Brown (69) have applied the spectrophotofluorometric technique of Duggan & Udenfriend to the measurement of vitamin B_6 in human whole blood With this method, only one ml of whole blood is required. Vitamin B_6 occurs in blood as pyridoxal and pyridoxamine.

Folic Acid

Studies in man of the prevalence and importance of folic acid deficiency in various disease states have been handicapped by the lack of satisfactory methods for the determination of the vitamin or its metabolites in body

fluids. The identification of the glutamic acid compound excreted in the urine of folic acid-deficient rats (70) as formiminoglutamic acid (FIGLU) by Broquist (71) and the demonstration of this substance in the urine of *folic acid-deficient human subjects, has led to the development of a satisfactory* method for confirming the clinical diagnosis of folic acid deficiency (71 to 75) Luhby, Cooperman & Teller (76) have increased the sensitivity of the urinary excretion test by administering an oral load of 15 gm of L-histidine monohydrochloride daily, in three divided doses, for two to three days before collecting the urine for the determination of FIGLU. Under these conditions, urine FIGLU levels do not exceed 30 µg. per ml. in non-*folic acid-deficient individuals; whereas they are three to 1000-fold greater* in the presence of folic acid deficiency

Microbiological determination of blood folic acid activity using *Lactobacillus casei* has reported by Toennies, Frank & Gallant (77) and, with *Bacillus coagulans* No 3084, by Baker, Hutner & Sobotka (78). Recently, Herbert *et al.* (79). using a modified *L. casei* technique, have demonstrated that the "folic acid activity" of sera thus determined serves as a reliable indicator of folic acid nutriture

Will *et al.* (80) maintained three of 36 patients with pernicious anemia for 10 years in a satisfactory hematologic and neurologic condition on folic acid therapy alone; however, the great majority of the 36 patients relapsed within four years, those who had had no previous liver extract or vitamin B_{12} therapy relapsing sooner. These workers were unable to find any evidence that folic acid changes the requirements of human beings for small amounts of vitamin B_{12}. They did obtain evidence that normal folic acid metabolism is dependent upon an adequate supply of vitamin B_{12}.

Giles & Shuttleworth (81) found megaloblastic anemia of pregnancy to occur in 2 8 per cent of all hospital confinements (North Staffordshire, Great Britain) *Anemias with a hemoglobin of less than 45 per cent were more* often megaloblastic than normoblastic Their patients responded to folic acid therapy Rachmilewitz & Izak (82), working in Israel, observed "low [illegible] of pregnant anemic women with a

who had suffered three episodes of hematologic decompensation within three years and who, on each occasion, has had a striking hematologic and clinical response to large doses of folic acid. They suggest that "the possibility of correcting 'relative deficiencies' of hematopoietic substances such as folic acid should be considered in patients with hemolytic anemias and superimposed bone-marrow failure"

Vitamin B_{12}

According to Chow *et al* (84), the absorption of orally administered vitamin B_{12} by healthy human subjects is increased by giving it in divided

doses and by giving it in solution rather than in a capsule. It is impaired by vitamin B_6 deficiency, by hypothyroidism (85, 86), and by the prolonged administration of many commercial intrinsic factor preparations (87) It is increased in pregnancy, in spite of low maternal blood levels which reflect fetal demand (85, 88, 89), and by the administration in combination with D-sorbitol (90, 91). The site of absorption appears to be the ileum (92). Booth & Mollin (92) found that in patients whose ileum had either been resected or short-circuited, the absorption of test doses of radioactive vitamin B_{12} was invariably subnormal and was unaffected by intrinsic factor or by previous treatment with chlortetracycline Evidence of B_{12} deficiency was found in many of these patients. Grasbeck, Kantero & Siurala (93), following through on the finding by Grasbeck & Nyberg (94) that calcium ions probably are necessary for the intestinal absorption of vitamin B_{12}, found that a deficiency of free calcium ions in the intestine is one of the causes of the poor vitamin B_{12} absorption in steatorrhea.

D-Sorbitol does increase the intestinal absorption of vitamin B_{12} by persons with gastric achlorhydria but it has little or no effect in patients with pernicious anemia (95, 96). Vitamin B_{12}-intrinsic factor preparations increase the absorption of orally administered vitamin B_{12} in pernicious anemia patients; however Killander (97, 98), and Schwartz, Lous & Meulengracht (99) have adequately demonstrated that in many pernicious anemia patients so treated a blockage to absorption eventually occurs The mechanism of this is unexplained but it may represent an immunity phenomenon (99) It must be concluded that oral therapy of pernicious anemia with vitamin B_{12}-intrinsic factor preparations cannot be relied upon to provide adequate maintenance for all patients.

For the intestinal absorption of vitamin B_{12} in doses considerably larger than physiological amounts, intrinsic factor is not necessary. Brody, Estren & Wasserman (100) obtained excellent results in 17 patients with oral doses of vitamin B_{12} of 50 µg. three times daily, however normal serum vitamin B_{12} levels were reached in only three patients. Similar results were obtained by Chalmers & Shinton (101) who used 100 µg of vitamin B_{12} daily, and by Hemsted & Mills (102) who also gave 100 µg of vitamin B_{12} daily by mouth. It seems, however, that in the management of pernicious anemia parenteral vitamin B_{12} remains the most dependable form of therapy Thompson & Hecht (103) have described a cyanocobalamin zinc tannate complex for parenteral administration, which has repository or long acting properties

It is becoming more and more apparent that the bone-marrow and peripheral blood changes of pernicious anemia are not necessarily early or constant components of clinical vitamin B_{12} deficiency. Jewesbury (104) has described five cases of subacute combined degeneration of the cord with "insignificant" changes in the peripheral blood and with normoblastic bone-marrow. Wiener & Hope (105) point out that the cerebral symptoms of

vitamin B_{12} deficiency may precede the appearance of anemia by years, and may occur in the presence of a completely normal blood picture and bone-marrow. Heaton, McCormick & Freeman (106) found the serum vitamin B_{12} levels to be abnormally low in 13 patients with tobacco amblyopia. Improvement followed treatment with vitamin B_{12}. They emphasize that, in heavy smokers, amblyopia may precede the development of other signs of vitamin B_{12} deficiency by many years. These observations are not in agreement with the conclusion arrived at by Darby *et al.* (107) that macrocytosis is the most sensitive indicator of vitamin B_{12} deficiency. From the long-term observation of pernicious anemia patients, Darby *et al.* (107) have estimated the minimal daily dietary requirement for vitamin B_{12} to be between 0.6 to 2 8 µg. Gräsbeck (108), however has calculated that the daily loss of vitamin B_{12} is approximately 6 µg, and he suggests that "one should administer so much vitamin B_{12} that 5 to 6 µg. are retained per day."

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PSYCHIATRY: BEHAVIORAL PROBLEMS IN THE ADOLESCENT[1]

By J Roswell Gallagher, M.D. and Herbert I. Harris, M.D.
The Adolescent Unit of the Children's Hospital
Children's Medical Center, Boston, Massachusetts

Introduction

In recent years there has been a sharp increase in physicians' interest in adolescents and a wider appreciation of the physiological and psychological differences among children, adolescents, and adults. As further evidence of this we now have this special review of the behavioral problems of this age group.

This greater interest arises partly from the fact that since many illnesses which formerly had a high mortality rate in little children and adults are now under better control, physicians may now give a higher priority to some of the problems of adolescence. Other factors are the increase in juvenile delinquency (which has focused much attention on adolescents), physicians' greater understanding of personality, behavioral, and psychosomatic disorders, and the increase in psychosomatic disorders which seems to have resulted from the stresses of present-day civilization. In any event, whether or not these are the causes, it is a fact that adolescents and their disorders are now receiving more attention than formerly Furthermore, there is a tendency to consider this age group's difficulties as distinct from those of little children or of adults Since their characteristics (and those of many of their illnesses) differ from those of little children, adults, and the elderly, it is proper to do so but, on the other hand, it is important to remember that this group's difficulties are often a reflection of the influences in their earlier lives and that their present medical management affects their future as well The advantages, and they are great, of the "age-group care" of psychological and medical disorders can be lamentably diminished if one focuses only upon a single period of life and does not give thought both to the patient's past and his future (1).

Recent Developments

Among many new developments in the field of adolescents' behavioral problems, five at once suggest themselves The first of these is the increase in teachers' understanding of the emotional factors in learning and the consequent greater utilization of guidance and mental hygiene clinics by educators. An American Public Health Association committee's report (2) which outlines suggestions for a secondary school's health program advised

[1] The survey of the literature pertaining to this review was concluded in June, 1959

that because of the importance of the emotional health, those directing secondary school health programs help to initiate and lend their aid to all efforts designed to promote a wider understanding of the effect of emotional factors upon learning, physical health and effective living . . . (and) that efforts which will provide those trained persons who are needed within a school, if it is to have a program which will promote a healthy development, be supported

Such principles, which suggest that teachers teach people, deviate considerably from the precept which held that a school's business was only to teach subjects.

Abrahamsen's investigation (3) of the number and kind of mental health facilities existing in schools, although indicating that mental health services and principles are yet far from ideally integrated in them, show that very considerable progress has been made Admittedly, it is unfortunate that in the 187 school systems his group surveyed 17 per cent had no mental hygiene services available, but only a few years ago it would have been considered amazing that 83 per cent of them did. The discouraging commentary of this survey is the dearth of professional personnel available for the jobs to be done; the encouraging features are the provisions which are being made for, and particularly the increased awareness of, the services which properly trained physicians and mental hygiene personnel (psychiatric case workers, psychologists, and psychiatrists) can offer schools.

The second new development is the establishment of special medical clinics for adolescents These are general clinics, caring for all of the disorders which occur in this age period and, although not encompassing guidance or mental hygiene, yet serve considerably to lighten the load of those overburdened psychiatric clinics These general clinics can adequately care for many of adolescents' minor behavioral, personality, and psychosomatic disorders as well as their strictly medical ones, and also may be able, since they are not psychiatric clinics, to help those adolescents who refuse to attend one Psychotherapeutic techniques in these clinics are carried out by general practitioners, internists, or pediatricians who usually have the benefit of a psychiatrist's advice and supervision Such clinics have recently been established in California (4), Colorado, the District of Columbia (5), Maine, Massachusetts (6); Ohio, Pennsylvania (7); and in medical facilities of the military services (8); and are already in operation or planned elsewhere. Not only because mental hygiene clinics are overcrowded and many young people reject their care, but also because many of their behavioral disorders can be cared for very satisfactorily by others than psychiatrists, it would seem wise, particularly if psychiatric supervision can be furnished, to extend these facilities. The *British Medical Journal* (9) has commented "the problems of youth are many sided and compelling To their solution medicine has a contribution to make: this is surely above all in the sphere of the family doctor"

The next significant development is in the college student mental health

area. Thirty years ago the late Clements C. Fry (10) was pioneering in this field and now, although only about 25 psychiatrists devote their full attention to this older adolescent group (11), the amount and quality of college student mental health work has vastly increased Farnsworth (12) has emphasized the importance of this work

> .. one of the major problems of our age is that of learning how to deal with the hostility and aggression in our peoples of all countries .. We see it now in the recurring crises that occur in international relations ... Therefore I believe that we must work through a group of people who are influential in changing attitudes, and there is no more influential group that I know than our college students.

Unfortunately, however, only a small portion of these physicians' efforts can be directed to this problem Henn (13) believes that the stresses imposed upon college students apparently result in a higher suicide rate than in non-student groups, and though few students have major psychoses, anxiety states are a relatively common complaint (14) No doubt a substantial proportion of those who find it necessary to drop out of college do so because of "personal distress, moodiness and transient instability which is so common as almost to be natural in this age group" (15). To combat this state of affairs and to insure the greater effectiveness of those who are intellectually superior, it is suggested that universities increasingly experiment "with modified techniques to give more latitude to those students whose maturation is not proceeding as smoothly as is normal," and give more consideration to individual differences (15)

Another new development concerned with adolescents' behavioral problems is the training of physicians in understanding and managing them. It is not enough to establish only those special clinics which have been referred to; they should be staffed with physicians who have a greater than usual knowledge of this age group's emotional disorders. The past few years have witnessed a greater interest among physicians in acquiring a psychotherapeutic approach to patients, not with an aim of becoming psychiatrists, but of enlarging their treatment so that it might more fully encompass a patient's emotional needs This approach, which Bibring (16) considers "a most helpful tool, providing insight into the patient and into oneself and increasing the doctor's skill within his own specialty by blending his medical knowledge with the understanding of the patient's personality and psychological needs," is certainly most desirable in the care of adolescents. Fortunately, a degree of familiarity with this approach sufficient to result in a considerable improvement in the physician's effectiveness with his patients is not prohibitively difficult or time-consuming to obtain Balint (17) has described in detail his training of general practitioners in the psychotherapeutic approach At present at least one formal postgraduate course (18), which "provides training in the care and understanding of adolescents and their ailments," is designed to offer general practitioners, internists, and pediatricians a greater familiarity with the emotional problems of this

age group. As a matter of fact, it is felt that this course, which includes training in adolescents' physical and emotional disorders,

> offers a unique opportunity to acquire skill in combining an understanding of a patient's characteristics and needs with a knowledge of his disorder when planning his management and care. . . . The adolescent, . . . overconcerned with himself, with this person he is desperately trying to develop, expects one to be as aware as he is of his need for success, for acceptance and to acquire independence His insistence that the physician pay attention to him, and the rewards one reaps when he does, offer a compelling and exceptional opportunity to supplement any physician's skill in dealing with people and to increase his tendency to treat them, not just the disease (19).

The final new development to be referred to here is that of special in-patient facilities for the care of emotionally upset adolescents. Despite the facts that in-patient facilities designed for adults or for little children are not appropriate for adolescents and that large numbers of adolescents have emotional disorders requiring hospitalization, for many years few new institutions were established for their care.

> Usually such boys and girls have either not been treated, or some agency has attempted to work out the best possible compromise plan. . . Some have necessarily been treated as out-patients (as in child guidance clinics for example), even though it is recognized that a proper type of residential treatment would be preferable . . . Many have been admitted to our psychiatric hospitals, where efforts have been made to provide maximum possible help. However, this has been usually in facilities planned primarily for the treatment of adults (20).

Recently the services previously offered by some state institutions and by such groups as the Devereux Schools have been supplemented by those designed exclusively for adolescents at the Menninger Foundation, the Kansas State Hospital at Topeka, the Neuropsychiatric Institute of the University of Michigan (20), and the Manville Residence and School in Boston. In-patient care facilities and research directed toward adolescents' more severe disorders are still, however, far from sufficient to meet the needs of this age group

Special Problems

Scholastic failure—Senn (21) has said that the most frequent reason for bringing children to guidance clinics today is not delinquency but learning difficulties and, although this may not be true of all clinics, failure to deal effectively with school work is certainly a major cause of the behavioral, personality, and psychosomatic disorders seen in psychiatric, medical, or student health services which deal with adolescents. In this connection it has been pointed out by Senn (21) among others that the somatic response to feelings which was utilized in childhood is likely, when similar circumstances again obtain, to be repeated during adolescence Under stress an adolescent acts like a child and, in particular, like the child he

was; if he is unable to meet adequately the stresses of school in childhood, we can, for instance, expect gastric symptoms at examination time in adolescence.

Much of the increase in those symptoms whose basis is scholastic failure is no doubt the result of such factors as the greater emphasis placed on education today by industry as well as by parents, the decline in both availability and acceptability of other than "white collar" jobs, and the prevalence of the concept that not only are all of our young citizens entitled to an education but that all are entitled to, and could profit from, the same kind of education; "too often we in America seem to mean that an equal education should be an identical education" (22). The concept of individual differences (21, 23, 24), which presents both administrative difficulties and seems to upset those who too literally interpret the idea of equality, could, if thoughtfully applied, prevent some of these disorders.

Frank (24), Farnsworth (11), Liss (25), Pearson (26), and Remmers (27) are among the many psychologists, physicians, and psychiatrists who, in addition to educators, have applied themselves to the causes and management of scholastic failure so that today we can more effectively both treat and hope to prevent these problems. It is agreed that the first step in the investigation of these young people is a careful medical examination with special emphasis on the status of the central nervous system including, vision and hearing; and next the level of such basic skills as reading, spelling, and arithmetic, and the level of intelligence ought to be evaluated. However, a study of attitudes and emotions is often the most fruitful area—, particularly when the patient is a high school or college student. Early discouraging, embarrassing, or frightening experiences in school, a poor parent-relationship (and a consequent absence of desire to become like or to please them), a preoccupation with sex, an inability to behave aggressively in school, or a fear of failure are among the many sorts of disordered feelings which adversely influence school performance in those who are intellectually able (28) Blaine (29) has classified the sources of college students' failure into six categories. The first is a preoccupation with such problems as an upset relationship with a loved one, the death of a friend or member of the family, or financial difficulties Another is the feeling that he is working in a void, that no one cares, that to work is to no purpose; studying to him or to her unappreciated by others or meaningless for its own sake is regarded as futile. A third source of failure is a need to fail because of the attention it brings; these are the young people whose best efforts never bring their parents' praise and only result in higher goals being set for them. Never allowed to have plans or goals of their own, some find that disappointing rather than pleasing their parents yields them attention Still another mechanism is that which involves hostility toward a parent; by failing in school the parent can be punished A fifth is a deep feeling of inferiority and the conviction that his or her ability is limited and could

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& Hobbs' (42) study of taxi drivers, which suggested that high accident rates and poor social and personal adjustment are correlated, has led to other studies of the personalities of those who are involved in many accidents Accident repeaters among members of the armed forces, many of whom were in or close to adolescence, have been found to have undesirable attitudes toward authority (43) and less capacity for controlling hostility, to be self-centered, and unable to tolerate tension (44). Though this problem deserves further investigation it would seem that inexperience and emotional factors play the major roles in this excessive motor vehicle accident rate; in most cases the adolescent has a higher physical and psychomotor efficiency than older age groups whose rates are low (40, 45)

Obesity—Bruch's (46) studies of obese adolescents have been outstanding, and have emphasized how important food can become in the minds of young people and how it can be used by them as a source of satisfaction and an avenue of escape She has ably pointed out the relation between obesity and the emotional disturbances which develop out of the normal processes of adolescence, the psychologically damaging effect of the fatness itself upon an adolescent's personality development, the adverse effects which may follow a single-minded therapeutic approach to the problem, and the exceedingly low frustration tolerance which these people have (47) Most important to remember are the danger and the futility of prescribing a reducing regimen unless previous attention is given to any underlying emotional difficulty. Further, to deprive these patients (by withholding food) before attacking the cause which has led them to seek satisfaction in food may well make matters worse.

However, despite the fact that at times emotional factors seem to be predominant in those who are obese, genetic factors, the role of activity (48), the effect of food's specific dynamic action or of glucose levels upon the hypothalamus and the appetite (49), and individual variations in the manner of utilizing and storing food eaten, should be kept in mind (50). Physiological as well as psychological factors need thorough consideration; an increase in the quantity and variety of the adolescent's activity is likely to be more effective than a concentration of attention upon food intake

Rebellion—The process of transition from dependence towards independence of family is one which often creates friction and misunderstanding in the home, anxiety in adolescents, and hostility in parents, yet it is one which must be made if a healthy, adult, emotional state is to be achieved (23, 29, 51). During this process adolescents vacillate between defiant, headstrong, rebellious, and at times even mature behavior, and childish, demanding ways insisting upon adults' help At times it seems as if they have a need to depreciate people, ideas, and things (28), or to upset a formerly pleasant home (29) in order that it may be easier to leave Dunbar (52) has stressed the postulate that various rebellious and delinquent patterns are used by adolescents in an attempt to restore emotional

not bring success; these young people do not try in order that this, not lack of intelligence, may be the obvious reason for failure. Finally, there are those whose fear of their ability to control their own aggressive impulses leads them to behave passively; they cannot express their well of energy and aggression constructively in their studies. Such devices as ventilation of concern, emotional support, an understanding of the motives of their own behavior and emotions, opportunities to gain confidence, and association with mature and stable adults whom they may wish to imitate and to please, will assist many of these young people to utilize their intelligence effectively (29).

A special reason for failure in school is specific language disability. This condition, which has been referred to as reading disability, congenital word blindness, dyslexia, etc, although a handicap in the early years of school, may not be recognized until the demands on reading and writing at the high school level, bring it to attention. It should be thought of in any instance of scholastic failure and particularly when the pupil's spelling and reading skill seem to be below the level one would expect of one of his intelligence and background (23, 28).

There is as yet no general agreement as to the cause of this condition Hallgren (30) believes that it follows a monohybrid, autosomal, dominant mode of inheritance, and Hermann & Norrie's (31) series of 11 pairs of monozygotic twins (both members of whom had typical signs of word blindness) and of 30 pairs of dizygotic twins (in whom these signs appeared in both twins in only six instances) lend further support to clinicians' impression that a genetic factor is involved Drew (32) has suggested that the disorder is a congenital disturbance of Gestalt function; and Hermann (33) sees a relation between it and Gerstmann's syndrome (which is characterized by right-left confusion, finger agnosia, acalculia, and agraphia) and believes these patients have an inherited disturbance in functions having to do with direction in space Zazzo *et al* (34) discuss the poor auditory and visual perception of sounds and symbols of these young people and their disturbance of spatial orientation.

In addition to the beneficial effects of such remedial methods as those outlined by Gillingham (35), more recently there have been preventive efforts by Gillingham (36), Spaulding (37), De Hirsch (38), Filbin (39), and others If, at least in those whose disability is not severe, such methods can prevent the frustration which intelligent children almost inevitably experience when learning to read and spell, much will have been accomplished

Auto-accidents.—Although all investigators do not agree, it seems likely that young drivers have proportionately more than their share of accidents (40), and it is a fact that in the 15- to 19-year age group, motor vehicle accidents are by far the most common cause of death (the rate was 34 5 per 100,000 in 1955, in contrast to rates of 7 9 from neoplasms, 6 1 from cardiovascular disease, 0 9 from all forms of tuberculosis) (41) Tillmann

Its statement of the problem is built upon the concept "that behavior and misbehavior always represent an interaction between the human organism and the cultural environment" Its first section examines many popular notions and misunderstandings concerning delinquency, another section reviews the problem in cultural and psychodynamic terms, and a third considers delinquency as a legal phenomenon, discusses methods for the early identification of delinquents and the school's role in educating and rehabilitating them. This group considers delinquency as representing adaptive behavior · "the delinquent act may often represent the only resolution the youngster can find to his personal social problem"

In addition to those very serious aspects of adolescent behavior, there are also the relatively minor but annoying matters associated with conduct at parties, suitable hours for parties, use of automobiles, etc. The Minnesota Teen-Age Code (58) is a valuable outgrowth of efforts to achieve better cooperation between adolescents and their parents in these matters The Code's success, and it has been considerable, has no doubt been attributable to the facts that young people themselves prepared the original draft and actively participated in the final one, and that it was subsequently accepted by an entire community, not by just a few families.

Psychotherapy of Adolescents

The increasing attention given to adolescents is resulting in a wider understanding of the differences between this age group's problems and treatment and those of little children and adults. Erikson (59), Josselyn (60), and Frank (24) and the references they cite remain excellent source material It is important to remember that although adolescents differ from other age groups, there are also differences within their age period itself; the 13-year old and the 17-year old have different needs, characteristics, and problems Geleerd (61) refers to three phases of adolescence: the first from about 11 to 13, the period of physiological maturation, the second from 14 to 16; and the third from 16 to 20. In early adolescence the boy or girl struggles to maintain the defenses of the latency period; in mid-adolescence there is conflict between the homosexual and heterosexual drives; and in late adolescence heterosexuality is well established Important as it is to remember these stages in adolescence and to evaluate and manage one's patients accordingly, it is equally necessary to keep in mind the fact that chronological age is of little value in categorizing adolescents (62). There is wide variation among those who are at the same chronological age; one 13-year old may think, appear, and behave as many do at 16, and similarly a 17-year old may more closely resemble one's concept of what a 13-year old is like.

Blos (63) has commented on the recapitulation of earlier patterns of behavior in adolescence, on the importance of the antecedents of adolescent behavior in determining its quality, and particularly upon the *relationship*

equilibrium, and Kaufman & Makkay (53), among others, have emphasized that rebellion is a stage in the normal growth process. When parents have not previously been overindulgent or rejecting, and when they do not treat rebellion with excessive intolerance, young people, during this period, are less likely on the one hand to become delinquent or on the other to become so guilty that the natural process of freeing themselves is curbed

Delinquency.—Though it may at times seem that juvenile delinquency receives a disproportionate amount of attention and overshadows both good behavior and also the need for attention to other problems of young people, it is obviously true that the high rate of juvenile delinquency here and abroad deserves even more than the considerable study and efforts already expended on it. As the Gluecks (54) have pointed out, it is not a problem for the law, medicine, sociology, government, or the church alone; its causes are multiple. Beck (55) has very succinctly reviewed the problem:

> Delinquency is . . . produced out of the variables of community, individual, and family behavior. It cannot be viewed as a disease entity in itself with a specific method of treatment or a specific means of prevention. . . . The specific preventive measures for psychopathic delinquency would be concerned with strengthening family life, eliminating unnecessary placement of children away from their homes, and providing good foster homes for youngsters who must be placed during early years. . . . On the other hand, measures of community reform aimed to reduce social delinquency would not have a marked effect on the production of neurotic delinquents. To prevent this latter type, measures addressed toward increasing parental abilities would be more appropriate . . . Children, however, may not be able to establish a satisfactory relationship between themselves and the world in which they live because of the social and moral climate of their communities, a climate not directly affected by the provision of good recreational, school, health, or social services . . . Thus to be effective, a program to combat delinquency must on the one hand have an impact on the values by which people live, tending to create a social and moral climate conducive to healthy development; on the other hand, it must promote the development of necessary programs to meet childhood needs

Even though he recognizes the value of psychological concepts in our attempts to understand and to combat juvenile delinquency, Miller (56) has urged that these concepts should not divert our attention from the relationship of the American lower class society to adolescents' criminal behavior He regrets our emotional resistance to the idea that there are classes within our society and the "pervasive" use of psychodynamic theory as the frame of reference for deriving the causes and cures of juvenile crime He regards these young people (and their heroes and associates) as fearless, sentimentality-scorning, excitement-seeking, combative, reckless, and as not fitting easily into an education-oriented, cooperative, physically unaggressive, orderly way of living.

The report of the National Education Association's Juvenile Delinquency Project (57) has attempted to present an integrated theory of delinquency

I take it that it is normal for an adolescent to behave for a considerable length of time in an inconsistent and unpredictable manner; to fight his impulses and to accept them; to ward them off successfully and to be overrun by them; to love his parents and to hate them; to revolt against them and to be dependent on them; to be deeply ashamed to acknowledge his mother before others, and, unexpectedly, to desire heart to heart talks with her; to thrive on imitation of and identification with others while searching unceasingly for his own identity; to be more idealistic, artistic, generous and unselfish than he will ever be again, but also the opposite. self-centered, egoistic, calculating Such fluctuations between extreme opposites would be deemed highly abnormal at any other time of life At this time they may signify no more than that an adult structure of personality takes a long time to emerge, that the ego of the individual in question does not cease to experiment and is in no hurry to close down on the possibilities .. I think that he should be given time and scope to work out his own solution. Rather, it may be his parents who need help and guidance so as to be able to bear with him There are few situations in life which are more difficult to cope with than an adolescent son or daughter during the attempt to liberate themselves

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REACTION TO SHORT-TERM RADIATION IN MAN[1]

By Herbert B Gerstner,[2] M D.

Department of Radiobiology, Air University School of Aviation Medicine, USAF, Randolph Air Force Base, Texas

So swift and so vast is the accumulation of information in the radiation field that only small segments of it can be covered adequately in a few pages; for the present review, the meaning of the title has been restricted as follows: "reaction"—those physical and mental changes which occur during the first three days after exposure, and which are detectable by clinical observation, interrogation, and routine laboratory procedures; "short-term" —exposure times of less than one hour; "radiation"—penetrating ionizing radiations in the form of hard conventional or supervoltage x-rays, gamma photons, and neutrons. After these restrictions, the problem to be discussed emerges as a well-known clinical entity—"radiation sickness" (1 to 3). This systemic reaction has lost its significance in radiotherapy since it can be avoided completely, or dwarfed to the role of an irrelevant side effect, by improved techniques involving both fractionation of dose and closer concentration of radiant energy in the tissue volume to be treated. The advent of the atomic age and possibility of large-scale nuclear disasters, however, demand renewed interest in early radiation-induced sequelae as potential cause of disability temporarily paralyzing exposed populations, and as a medical problem of concern to all physicians who may have to handle radiation casualties at emergency stations. The atomic age also necessitates more precise terminology based upon the various phases in the clinical response to penetrating radiation To specify the early burst of signs and symptoms, the broad term "radiation sickness" should be replaced by the more restricted and more striking denotations "initial reaction" or "prodromal reaction" (4); subsequently, these two terms will be used interchangeably. The present review, then, presents the initial reaction to short-term, penetrating radiation as it emerges from radiotherapy, nuclear accidents, and Japanese bomb casualties.

Typical Picture

Although displaying colorful pictures brought forth by wide variability in both time course and severity, the initial reaction possesses a definite basic pattern which can be established with reasonable accuracy.

Clinical observations.—Irradiation with several hundred roentgens is not accompanied by sensations; therefore, in the absence of light and noise

[1] The survey of the literature pertaining to this review was concluded in April, 1959

[2] The opinions expressed in this article are those of the author and are not to be construed as representing official USAF policy.

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tion value, is largely a result of a rise in neutrophil count; the lymphocytes do not contribute materially to this early reaction It should be emphasized that in a high percentage of cases the hematologic alterations are the first detectable changes clearly preceding other objective and subjective manifestations of the prodromal phase [Court Brown (6)]. The initial leukocytosis—showing wide individual variability, little relation to dose, and no "shift to the left" in maturity of cells—generally is a very transient event of a few hours' duration; occasionally, however, it may last longer and may extend even into the third postexposure day. After having passed through the leukocytotic phase, the granulocytes become relatively stabilized at their normal level for about five days; thereafter, they begin to participate gradually in the typical radiation-induced leukopenia (6, 7). The lymphocytes, without essential contribution to the initial leukocytosis, decrease in number as early as the first 3 days after irradiation, while the thrombocytes remain unchanged (6 to 8).

Physical examination reveals a tendency toward slightly elevated temperatures, mild hypotension, and an accelerated pulse rate. These changes, when they occur at all, are of moderate degree over a wide dose range; but they may become sharply accentuated, occasionally in rare cases of extreme radiosensitivity and, consistently, in persons exposed to very high doses of several thousand roentgens to the entire body. Under these circumstances a serious picture of circulatory collapse may ensue.

Gradation of reaction—Despite difficulties residing in the wide variety of individual pictures, practical considerations require establishment of [illegible]

many other kinds of indisposition, particularly motion sickness with which they also have another common trait—a strong dependency on psychogenic factors [Gauss & Lembcke (9); Béclère (10)] Starting at several hours postexposure and proceeding in waves created by a two-day succession of exacerbations and remissions, they seldom appreciably impair physical and mental faculties Thus, these forms probably are irrelevant to both radiotherapy and civil defense Quite a different appraisal applies to moderate forms. Here, between 2 and 4 hr after irradiation, the exposed person enters a phase characterized by the combination of marked weakness with severe nausea and vomiting. During that phase, generally lasting 5 to 8 hr., physical as well as mental capabilities definitely are reduced; thereafter, of course, insignificant remnants of the reaction linger for several days. Obviously, the brief phase of disability represents a trifling effect in radiotherapy patients but will require attention when it afflicts persons whose occupation demands full possession of physical and mental alertness, e g , aircrews, drivers of automobiles under emergency traffic conditions, and executive officers who must render swiftly both sound judgment of a given situation

phenomena, the event may pass completely unnoticed. Such exposed persons remain *perfectly normal and asymptomatic during 1 or 2 hr.* postirradiation when growing fatigue is experienced Typical radiotherapy patients then cease their usual activities— walking about the ward, talking with friends, and reading. Lying on the bed, they become less and less interested in their environment as time passes This change is particularly conspicuous in extroverts who, normally, are the life of the ward; they become quiet, withdrawn, and asocial. Often, the condition is described as "washed-out" or "worn-out." Occasionally, listlessness and lack of initiative progress to severe apathy, extreme weakness, or even prostration. Not infrequently, this fatigue complex is accompanied by an emotional disturbance in the form of acute mental depression leading to spells of hopelessness and despair. Simultaneously, the patient complains of headache of uncharacteristic distribution, insomnia, dullness, dizziness, and sometimes even of vertigo.

Concomitant with apathy and mental depression, a more or less severe gastrointestinal distress develops. Between 1 and 2 hr. postexposure, the disorder starts with complaints of an "upset stomach" and loss of appetite. Nausea, retching, and frank vomiting soon supervene and increase in intensity until a climax is reached somewhere between 5 and 8 hr. postexposure. At that time, the combination of fatigue and gastrointestinal distress may lead occasionally to a serious shocklike picture. Thereafter, the systemic reaction recedes almost as fast as it developed On the second day, fatigue and the episodes of nausea, or even of vomiting, may still persist; however, the general condition is markedly improved. On the third postexposure day, most of the patients become asymptomatic, and only a few exceptional cases continue to show fatigue, anorexia, and mild nausea as remnants of the vanishing initial reaction. Generally, the sequence of disappearance is as follows: vomiting first, followed by nausea, then fatigue, and finally anorexia

Thus, the initial reaction can be conceived as composed of two complexes characterized, respectively, by fatigue and vomiting These complexes appear to be relatively independent of each other. Occasionally, fatigue is the only complaint More frequently, fatigue is the predominant basic symptom *upon* which, as fleeting events, nausea and vomiting are superimposed. Generally, concurrent and full development of the two complexes is observed. Finally, as a rarity, only bouts of nausea and *vomiting may occur without any manifestation of the fatigue factor.* This relative dissociation between "fatigue and vomiting complexes" perhaps indicates that the prodromal reaction is caused by two separate etiologic processes as suggested by Court Brown (3, 5).

Special observations.—Among laboratory findings, changes in the blood picture are especially outstanding, consistent and unambiguous *Most* conspicuous is a leukocytosis beginning within a few hours of postexposure. The increase in white cells, which frequently leads to a doubling of the preradia-

nature. Conversely, the presence of prodromal effects points to a dose in excess of 100 r and, thereby, to the likelihood of significant clinical manifestations during the later phases of the acute radiation syndrome [Gerstner (11)].

Dose-severity pattern—Close to the threshold dose of 100 r the initial reaction, if any, predominantly proceeds in mild forms characterized by *brief spells of fatigue, anorexia, and nausea* But when the dose approaches 200 r, approximately the following distribution of severity emerges from the therapy data reported by Miller *et al* (7) and by Levin *et al.* (12). completely asymptomatic, 20 per cent; mild reactions not exceeding nausea, 20 per cent; moderate reactions with vomiting and marked weakness, 50 per cent; and severe reactions leading to profuse vomiting and prostration, 10 per cent The fact that Brucer *et al.* (13) observed a similar distribution after accidental exposure to distinctly higher doses probably indicates two mechanisms first, because of a spontaneous tendency toward nausea, advanced cancer patients react to equal amounts of radiation somewhat more vehemently than healthy persons; second, the distribution pattern fails to undergo drastic changes as the dose exceeds the 200 to 300 r range The second point finds increasing support in evidence derived from leukemia patients who received high total-body doses prior to bone-marrow transplantation [Thomas *et al* (15); Andrews (16)] At approximately 300 r, each individual person seems to display fully the severity of the initial reaction peculiar to his degree of susceptibility; up to 600 r, perhaps even 800 r, no appreciable further increase in severity ensues This surprising dose-effect relationship probably can be explained as follows· not unlike susceptibility to foreign protein, radiosensitivity—with respect to prodromal effects—varies widely among a population; hypersensitive individuals are characterized by low threshold dose and violent reaction, *conversely*, hyposensitive persons are typified by high threshold and mild response According to this inherent property, the prodromal reaction proceeds with a certain degree of severity, irrespective of the amount by which dose exceeds the fully triggering value (300 r). Most likely, the drastic difference in individual sensitivity is typical for the initial reaction only, but not for the subsequent damage to the hematopoietic system or gastrointestinal tract; consequently, prognostic predictions made from the severity of the former to the degree of the latter are dubious As support for this statement, two examples may suffice First, after the Y-12 accident, the person exposed to the second highest dose (339 rads) remained completely asymptomatic during the day of exposure but passed through a severe bone-marrow depression several weeks later [Brucer *et al* (13)]. Second, as illustrated in Table I, the incidence of typical radiation-induced vomiting among the Hiroshima population decreased with dose since exposure groups were designated and composed according to both distance from hypocenter and the degree of shielding afforded by interposed structures (the dose received

and proper decisions about countermeasures. To what extent, in analogy to motion sickness, clinical manifestations can be suppressed by strong motivation and will power remains unsolved. As revealed by numerous case reports, evacuation of the disaster area in Hiroshima was certainly hampered by moderate forms of the reaction; understandably, no information is available about instances, if any, in which disability assumed such a degree as to prevent escape from the spreading fires The latter point also applies to the occasional severe forms which were characterized by profuse vomiting and prostration. Between 2 and 4 hr postexposure, complete incapacitation commences and persists throughout a period of 5 to 10 hr. Without assistance, such persons obviously are doomed in disaster situations, and their number among Hiroshima casualties will forever remain a matter of conjecture That distinction of three grades in the prodromal reaction is important not only for civil defense considerations but for other fields as well, may be exemplified by their therapeutic management: mild forms, psychologic reassurance; moderate forms, psychologic reassurance combined with antiemetic medication; and severe forms, parenteral supply of antiemetics, fluid, and electrolytes [Miller *et al.* (7); Gerstner (11); Levin *et al.* (12)].

Dose Dependency of the Initial Reaction

The relationship between radiation dose and degree of prodromal reaction is of the utmost practical importance In nuclear disasters, accurate results of physical dosimetry may not be available early enough to form the basis for immediate action and, thus, the clinical picture must serve temporarily as both indicator of exposure and guideline for countermeasures [Brucer *et al.* (13)] What conclusions about the dose can be drawn from severity, incidence, and time of onset of the prodromal reaction?

Dose-incidence pattern.—Obviously, knowledge of both physical radiation factors and clinical reactions is requisite for predicting the dose-incidence pattern in large populations Since highly accurate information such as is now available refers only to whole-body therapy patients whose number decreases rapidly as the dose exceeds 200 r, this dose level presently forms the border line beyond which reasonably confident statements are increasingly superseded by conjectures based on scanty therapy and reactor accident data. Uncertainties, which increase with rising dose, apply not only to this paragraph but to the succeeding two as well Below 100 r, clear-cut prodromal reactions are not expected to occur in an exposed population [Glasstone (14)]. When the dose surpasses that threshold level, the incidence grows quite rapidly as shown by the following estimate: 150 r, about 50 per cent; 200 r, approximately 80 per cent; and 300 r, almost 100 per cent Thus, the absence of prodromal effects among an exposed population is of eminent prognostic significance; it indicates, with high probability, an air dose of less than 100 r and a subsequent clinical course of trivial

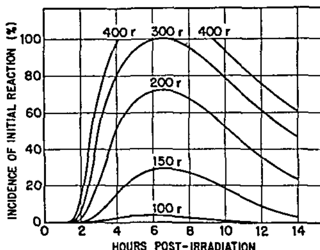


FIG 1 Estimate of incidence and duration of severe nausea, with vomiting in most cases, among a large population exposed to various air doses of penetrating ionizing radiation.

Dose and length of delay period—For two reasons, the greatest practical importance must be attributed to the characteristic time gap interspersed between irradiation and abrupt onset of the prodromal reaction. First, this brief delay period during which exposed persons still possess full physical and mental power, *may represent the only chance for active escape from* a nuclear disaster area. Second, it has been claimed repeatedly that the length of the time gap allows for the making of valid conclusions concerning both seriousness of exposure and prognosis of the subsequent acute radiation syndrome, allegedly, the earlier nausea and vomiting occur, the more severe is the ensuing disease To allow objective determination of this important factor, all available information has been summarized in the distribution pattern of Figure 2, showing the onset time of prodromal reactions in 97 persons who were irradiated either therapeutically or accidentally; *criterion for the inclusion of data has been reasonably accurate* attestation of the time when either severe nausea or, in most cases, frank emesis commenced Each mark, referring to one patient, indicates both the type of exposure and origin of data in the following manner: Members of a first group who received limited field x-radiation are represented by circles containing black segments wherein segment size reflects literature references as follows—three-quarters (18), two-quarters (19), and one-quarter (20). Members of a second group who received whole-body x-radiation are represented by circles containing black centers and references are denoted by the number of circles—one circle (7), and two circles (12). Finally, members of a third group, who suffered from short-term exposure to penetrating

TABLE I

INCIDENCE OF VOMITING DURING DAY OF ATTACK AMONG VARIOUS EXPOSURE GROUPS IN HIROSHIMA [COMPILED FROM OUGHTERSON (32)]

Exposure Group	Total Number of Persons	Incidence of Vomiting	
		Number of Persons	Per Cent
A	570	183	32.1
B	1119	272	24.3
C	1817	128	7.0
D	1604	59	3.7
E	711	17	2.4
F	575	14	2.4
G	267	1	0.4

by the group lessening as the identifying letter advances in the alphabet). Although data about prodromal effects in Japanese bomb casualties require careful scrutiny, two points in Table I are obvious beyond doubt: Even in the highest exposure group (A), the incidence of vomiting falls far below 100 per cent; yet, most members of this group subsequently suffered from severe forms of the acute radiation syndrome. In the low exposure groups (E-G), incidence does not reach zero despite the fact that the succeeding hematopoietic disturbance, if any, remained clinically insignificant. Collectively, this evidence strongly corroborates the view that, in the several-hundred-roentgen range, severity of prodromal reactions largely reflects individual susceptibility and, therefore, allows few prognostic conclusions about degrees of later clinical sequelae.

Dose-incidence-time pattern.—By taking into account even the mildest forms of reaction, and by adopting the rather dubious assumption that beyond 300 r practically everybody experiences some degree of early effects, an estimate of the dose-incidence-time pattern has been made which is graphically represented in Figure 1 This drawing, although conjectural in many details, depicts the following essential and rather well-established characteristics of the prodromal reaction A delay period ending with the sudden onset and build-up of the disturbance; a maximum of incidence among the population which lies somewhere between 5 and 8 hr. post-exposure, irrespective of dose, and a swift decline that becomes evident after the reaction has passed through the climax Because of the gradual ebbing of waves created by the succession of exacerbations and remissions, the duration of the entire prodromal phase is ill-defined; generally, emeses disappear after the second day. In a series of 33 unshielded Hiroshima survivors who were exposed to doses probably ranging from 400 r to more than 600 r, vomiting lasted, on the average, 40 hr. while nausea had a mean duration of 48 hr. [Clark & Lynch (17)].

as a nonspecific systemic response to sufficient intensities of any kind of penetrating radiation passing either through the entire mass of the body or through a fraction of its volume only; within a wide dose range, the time of onset and degree of response are predominantly determined by individual susceptibility. If a short-term dose of penetrating radiation is high enough to evoke prodromal reactions at all, the disturbance will begin between 1 and 5 hr. postexposure with a probability exceeding 90 per cent Later onset is suspect as reflecting a psychogenic response or some other complication Earlier commencement indicates either a pure psychogenic response or an overwhelming dose of several thousand roentgens.

INFLUENCE OF BODY REGION, AGE, SEX, AND OTHER BIOLOGIC FACTORS

One of the most outstanding features of the initial reaction to penetrating radiation is its startling dependency on "individual susceptibility" Obviously, it would be advantageous to both radiotherapy and nuclear power utilization if patients or personnel, before actual or potential exposure, could be screened according to this criterion By attempting to unmask the concrete factors behind the vague term "susceptibility" a basis may be found for answering the questions: which persons are hypersensitive, and which ones are refractory?

Body region—When applied to various regions of the body, equal doses elicit prodromal reactions which are identical in kind but different in degree; hence, ease with which the distress is evoked depends on the irradiated topographic area This regional difference is interesting scientifically because of its bearing on pathogenesis of the distress and, practically, because of its relations to the following facts: in nuclear accidents, homogeneous whole-body exposure often will be an exception rather than a rule; whereas, because of shielding by interposed structures, partial exposure, or at least inhomogeneous dose distribution, will predominate. In regard to sensitivity, radiotherapy experience with many thousands of patients has established firmly an order of gross body areas Points of general agreement can be formulated briefly as follows: from a peak in the epigastric region, sensitivity falls off toward the head as well as toward the thighs (20, 26 to 30); statistical comparison of various data discloses the quantitative relationship to correspond quite well with that expressed by Groedel & Lossen (26), namely, the same dose that induces prodromal reactions in 50 per cent of the patients when applied to the abdomen, causes the following incidence elsewhere: thorax 33 per cent; head and neck 25

0 per c

sensiti

for the drawing of definite conclusions; too often differentiation is impossible between findings chiefly reflecting preconceived pathogenic hypotheses and those originating in facts Therefore, it suffices to quote only one example which appears relatively well founded From observations made on

radiation during nuclear accidents, are represented by circles with letters indicating the corresponding reference—A (21), B (22), C (23), D (13), E (24), and F (25). Despite its unbiased selection, the data in Figure 2 form an impressive pattern in which, irrespective of their origin, 95 of 97 markings are arranged rather evenly between 1 and 4½ hr. postexposure. Inescapably, this striking feature leads to several important conclusions. First of all, onset time of the prodromal reaction is influenced very little by the size of the radiation field—be it as small as the pelvic area (19), or

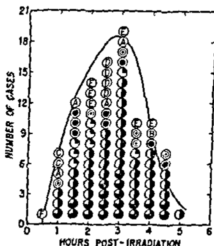


FIG 2 Distribution pattern of 97 patients which shows the time elapsing between end of radiation and onset of severe nausea associated, in most cases, with vomiting Further information is given in the text

as large as the entire body (7, 12); thus, dose distribution is precluded as an important factor Second, lack of separation between radiotherapy markings and nuclear accident symbols indicates irrelevance of radiation type —roentgen, gamma, neutron, or any mixture of these penetrating rays Third, even though the doses vary widely within the several-hundred-roentgen range, this variation barely affects the distribution pattern; hence, dose plays an unexpectedly minor role Although, by statistical means, a slightly falling trend of the delay time has been demonstrated for increasing amounts of radiation by Court Brown & Abbatt (19), this reduction becomes obvious, in single persons, only after exposures in the several-thousand-roentgen range (symbol F in Fig. 2) Evidently, after rejection of field size, type of radiation, and dose as major influences, it becomes necessary to search for another and a much more powerful agent as the determinant of the distribution pattern in Figure 2. This factor, almost certainly, is individual susceptibility.

Comment.—The preceding consideration reveals the prodromal reaction

the lower part of Table II. When the discussed limitations are taken into account, the Hiroshima data agree with radiotherapy experience in establishing a correlation between increasing age and decreasing proneness to prodromal reactions.

Sex.—Although Miescher (20) concludes from a comparison of 52 male with 69 female radiotherapy patients that the incidence of "radiation sickness" shows no significant sex difference, later investigators report for women a definitely greater proneness to prodromal reactions [Anzilotti (29); Pfahler (33)]. The existence of such a difference between

TABLE III

INCIDENCE OF VOMITING DURING THE DAY OF ATTACK AMONG 20-DAY HIROSHIMA SURVIVORS SEPARATED ACCORDING TO SEX [COMPILED FROM OUGHTERSON (32)]

Exposure Group	Number of Persons Observed		Persons Vomiting			
			Number		Per Cent of Observed Group	
	Male	Female	Male	Female	Male	Female
A	414	156	109	74	26.3	47.4
B	560	559	119	153	21.3	27.4
C, D	1704	1717	72	115	4.2	6.7
E, F, G	738	815	10	22	1.4	2.7
Total	3416	3247	310	364	9.1	11.2

sexes is confirmed beyond any doubt by the Hiroshima data compiled in Table III. Large sample size and consistency of trend—reflected in higher female values through- [illegible]

to be higher than that in men exposed to the same dose

Constitution.—Although the biologic factors discussed above affect susceptibility to some extent, their influence is much too weak to explain the wide individual variation displayed by the prodromal reaction. Search for a more potent principle necessarily leads to an inquiry about the role of specific constitutional traits. Thus far, attempts to establish correlations between the degree of susceptibility and certain anthropologic body types have been unsuccessful [Anzilotti (29)] General agreement, nonetheless, exists about the following fact: prodromal reactions with relatively low threshold doses and vehement character occur in persons classified either as neurasthenic (20), or as psychoneurotic (27), or as nervous and high-

400 patients, Anzilotti (29) derived the following order of decreasing radiosensitivity for abdominal regions: epigastric, hypogastric, periumbilical, splenic, renal-adrenal, and hepatic.

Age.—Radiotherapy experience can be summarized briefly as follows: comparison between young and old persons subjected to the same dose suggests for the first group a somewhat greater proneness to prodromal reactions; hence, radiosensitivity seems to decline gradually with advancing years. So slight is the effect, however, that this statement can be based only on clinical impressions, not on definite proof (20, 29, 31). It becomes obvious that the suggested trend is actually true when the Hiroshima data in Table II is subjected to the following scrutiny: several undersized subgroups—as small as 3 members—discredit the figures quoted for the youngest age range (0–4 years); consequently, these dubious values must be eliminated from further consideration In sharp contrast, the remaining figures can be accepted with the high degree of confidence arising from the data found in sample sizes of 25 to 2300 persons. According to the reliable part of the data, the incidence of vomiting clearly decreases with progressing age That this age relationship concerns a true radiation-induced reaction is affirmed by the fact that the falling trend manifests itself most conspicuously at high doses. in Table II, the dose lessens as the identifying letter of each exposure group advances in the alphabet It appears reasonable to assume that persons comprising exposure groups A and B fall into the several-hundred-roentgen range and, as a consequence, display relatively pure radiation sequelae Conversely, as the dose declines through groups C to G, radiation progressively loses its leading etiologic role to competing mental and physical stresses inherent in such large-scale disasters; at these low exposure levels, therefore, vomiting cannot be related precisely to the radiation insult as such This fact obviously explains the irregularities in

TABLE II

EFFECT OF AGE ON THE INCIDENCE OF PRODROMAL REACTIONS AMONG A SAMPLE OF 6613 HIROSHIMA 20-DAY SURVIVORS. TABULATED IS THE PERCENTAGE OF INDIVIDUALS OF EACH SUBGROUP WHO REPORTED VOMITING ON THE DAY OF ATTACK [COMPILED FROM OUGHTERSON (32)]

Exposure Group	Age Range in Years				Total
	0–4	5–14	15–49	50–100	All Ages
A	33 3	40.7	33.1	28.0	32.1
B	16 0	32 4	26 1	11 4	24.3
C, D	3.8	9 1	4.9	5.1	5 5
E, F, G	1 5	2 6	1.7	2 1	2.1

the lower part of Table II. When the discussed limitations are taken into account, the Hiroshima data agree with radiotherapy experience in establishing a correlation between increasing age and decreasing proneness to prodromal reactions.

Sex.—Although Miescher (20) concludes from a comparison of 52 male with 69 female radiotherapy patients that the incidence of "radiation sickness" shows no significant sex difference, later investigators report for women a definitely greater proneness to prodromal reactions [Anzilotti (29); Pfahler (33)]. The existence of such a difference between

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	Male	Female	Male	Female	Male	Female
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C, D	1704	1717	72	115	4.2	6 7
E, F, G	738	815	10	22	1 4	2 7
Total	3416	3247	310	364	9 1	11 2

sexes is confirmed beyond any doubt by the Hiroshima data compiled in Table III Large sample size and consistency of trend—reflected in higher female values throughout all exposure groups—bestow upon the findings a degree of confidence that renders further discussion redundant. In summary, this brief survey reveals the incidence of prodromal reactions in women to be higher than that in men exposed to the same dose.

Constitution—Although the biologic factors discussed above affect susceptibility to some extent, their influence is much too weak to explain the wide individual variation displayed by the prodromal reaction Search for a more potent principle necessarily leads to an inquiry about the role of specific constitutional traits. Thus far, attempts to establish correlations between the degree of susceptibility and certain anthropologic body types have been unsuccessful [Anzilotti (29)] General agreement, nonetheless, exists about the following fact: prodromal reactions with relatively low threshold doses and vehement character occur in persons classified either as neurasthenic (20), or as psychoneurotic (27), or as nervous and high-

strung (10, 34, 35), or as emotionally unstable (29, 36); in short, in persons characterized by lability of the nervous system, especially of its autonomic part. As yet, this "nervous imbalance" has been described too vaguely to allow further analysis, and objective measurements of the peculiarity have not been attempted. Frequently, "nervousness" may represent only the reflection of another systemic disturbance accessible to accurate appraisal. It appears certain that, eventually, such specific characteristics in a person's biochemical and physical makeup will make objective and replace his "traits" and, by doing so, will furnish also a solid foundation for the understanding of susceptibility to radiation.

Meal schedule.—Occasionally, exposure of the fasted patient—"on an empty stomach"—has been recommended for alleviation of "radiation sickness." Incrimination of food uptake as a precipitating factor probably results from the following fact: through its wavelike course, the prodromal reaction is capricious and unpredictable—presenting brief periods of relative well-being alternating with spells of severe distress Sometime, exacerbations can be traced apparently to meals or disagreeable sensory impressions; often, their cause remains completely enigmatic. Presumably, by eliminating some of the upsetting influences, an empty stomach might be of benefit; however, such a beneficial effect lacks sufficient clinical evidence. On the contrary, overwhelming experience favors maintenance of normal meal schedules and administration of the radiation treatment during one of the intervals—usually at 1 to 2 hr. following breakfast or lunch (12, 20, 37, 38) Under such a regimen the systemic reactions definitely are milder

Comment—Although several discrete factors have been isolated and assessed as contributors to the complex (radiosensitivity), they throw only partial light upon the wide variation found among persons, and, to a large extent, the problem still remains unsolved. Known facts can be summarized briefly as follows with a minimum of integral dose, the person most likely to develop a prodromal reaction is a fasted young woman, nervous and high-strung, receiving penetrating radiation to the epigastric region

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HIGH VOLTAGE RADIATION THERAPY[1]

By Morton M. Kligerman, M.D.
Department of Radiology, Yale University School of Medicine
New Haven, Connecticut

INTRODUCTION

In the past two decades, the practice of radiotherapy has seen strong development along three major lines. These include the widespread use of radioactive isotopes in investigational and clinical work; the rapidly increasing interest in the biologic effects of radiation; and the development of external radiation therapy equipment which produces gamma rays, x-rays, and electron beams in the supervoltage range. Although the number of supervoltage units was limited to a relatively few institutions before the end of World War II (10, 15, 16, 37, 50) this aspect of radiotherapy drew a great deal of attention in this early period. Following that war, numerous instruments were designed. Widespread clinical use soon became a reality, especially with the development of relatively inexpensive cobalt^{60} sources. This discussion will center mainly on the clinical aspects of the use of supervoltage radiotherapy, although a brief description of available apparatus will be included.

It is not too early to point out that the advantages of supervoltage therapy depend entirely on the physical characteristics of the beam. Clinical biological considerations have not been altered by the introduction of this equipment. The indications for radiotherapy, past and present, are determined by: a knowledge of the relative radiosensitivity of particular tumors and the normal structures in which they are located; knowledge of the direction, time, and extent of the spread of a particular tumor; and, the complications most apt to arise as a result of treatment. These considerations can best be expressed by saying that, in importance to the patient, knowledge of the natural history of the particular cancer and its sensitivity or responsiveness to irradiation far outweighs the availability of a supervoltage generator. Neglecting an adjustment in optimum tumor dosage between routine deep therapy equipment (200 to 250 kv.) and the supervoltage units, no appreciable change in the biologic response of tumor and normal tissues has been observed. Nevertheless, certain advantages of supervoltage radiation widen the horizon for the application of ionizing radiation in the treatment of the cancer patient.

ADVANTAGES OF SUPERVOLTAGE RADIATION

X-rays and gamma rays generated with an energy above one million electron volts (1 Mev) are considered to be in the supervoltage range. Equipment now available and in use includes instruments which develop

[1] The survey of the literature pertaining to this review was concluded in June, 1959

energies up to 70 Mev. The characteristic of the supervoltage beam as opposed to orthovoltage (200 to 250 kv.) is attributable to the forward scatter of the secondary electrons produced as the result of the interaction of the high-energy x- or gamma rays with the tissues. Like the primary x-ray beam, these electrons cause ionizations within the tissues. In routine deep therapy apparatus, however, the electrons so emitted scatter widely. As a result, the maximum dose in orthovoltage treatment is on the surface of the skin while with the forward scatter in supervoltage equipment, the highest dosage is found at some distance below the skin surface. This distance increases with voltage. Furthermore, side scatter is not appreciable and therefore little energy is dissipated beyond the cone of irradiation subtended by the primary beam. Because of the skin-sparing effect of supervoltage radiation, the tumor dose can be increased and indeed is essentially independent of the limitations which were formerly imposed by the skin. The lack of lateral scatter and high-depth dose decrease the total amount of irradiation absorbed by the patient and greatly reduce the incidence of radiation sickness

The improved physical characteristics of these higher energy beams permit utilization of rotation therapy with considerable advantage [Trump *et al.* (60); and others (5, 8, 22, 43)] This reduces the amount of radiation delivered to the normal tissues surrounding the tumor-bearing volume to a minimum (6, 18, 19, 36, 40, 47, 59, 63).

APPARATUS AVAILABLE

Gamma rays and x-rays are electromagnetic radiations of extremely short wavelength capable of passing through gaseous, liquid, and solid material, and capable of ionizing these substances. By convention, gamma rays refer to those electromagnetic radiations which are produced by the disintegration of the nucleus of radioactive materials, either occurring naturally or produced artificially. X-ray, on the other hand, is the term applied to electromagnetic radiation produced by electrical generators Gamma rays and x-rays of similar energy or voltage have exactly the same characteristics as far as their interaction with matter is concerned.

The disintegration of a particular radioactive material causes the emission of radiation of a limited number of wavelengths. For example, radioactive cobalt^{60} produces one photon of 1 7 Mev and one photon of 1 3 Mev The beam is relatively monochromatic Electrical generators produce photons through a whole spectrum of energy values with the highest energy being equal to the rated capacity of the machine For example, a 4-Mev linear accelerator produces photons of many wavelengths of which the shortest and most penetrating corresponds to an energy of 4 Mev. In general, the average energy of the beam of an electric generator is equal to one-half the maximum voltage A 3-Mev electric generator, therefore, would have an average energy of 1 5 Mev which corresponds to the energy of the cobalt^{60} beam (1 3 and 1 7 Mev) All x-rays are produced by the

bombardment of a target metal of high atomic number (tungsten, gold) by electrons which are accelerated to great speed, thereby increasing their mass. The 1- and 2-Mev resonant transformer generators have proved to be extremely reliable instruments (12). Also in this range is the popular 2-Mev Van de Graaff generator which produces its high voltage electrostatically (58). By removing the target of this instrument and adding a suitable auxiliary apparatus, the electrons themselves have a clinically effective penetration in tissue of approximately 0 6 cm

Linear accelerators are available that develop voltage from 4 to 70 Mev (23, 24). These instruments are very readily converted from x-ray beams to electron beams and have a high output of energy in either form The betatron (35, 41, 42), using a different system of accelerating electrons, can be utilized as either an x-ray or electron beam source and is available in energies from 22.5 to 35 Mev. For energies above 50 Mev, a modification of the betatron known as the synchrotron is more efficient (46, 52)

Perhaps the most popular supervoltage instrument is the radioactive cobalt^{60} teletherapy unit (4, 26, 39). Watson (63) lists the advantages of the cobalt unit over a 2-Mev electric generator as. (*a*) lower initial cost, (*b*) lower upkeep; (*c*) compactness and maneuverability; and (*d*) a monochromatic beam The disadvantage is the penumbra, or zone of reduced irradiation around the primary beam which increases the total amount of irradiation which the patient receives A second disadvantage is that these cobalt sources must be replaced every three years in order to keep a reasonable number of patients under treatment It should be pointed out that the popular cobalt units are relatively small and are used in rotation therapy with relatively short target-skin distances Although the skin-sparing effect is maintained, the depth dose is considerably reduced by this altered geometry as compared to the large cobalt units and electric generators. With the short focal distance the penumbra is large and, as a result, a high dose is delivered to the tissues surrounding the tumor This is often as great as the patient would receive during rotation therapy with a 250-kv. machine (43).

Treatment with heavy particles by means of the cyclotron (56) as well as the use of neutrons from the atomic pile is undergoing investigation (25).

It is important to appreciate that the differences between the several supervoltage machines, other than the last two mentioned, are small when compared to the differences between supervoltage units as a group and deep therapy units

GENERAL CLINICAL CONSIDERATIONS

Supervoltage radiation with its ability to spare the skin and deliver a high tumor dose has permitted two important advances First, it has increased the diameter of a tumor of any particular type which can be accepted for definitive treatment with hope of long-term survival. This means

that a greater number of patients can be subjected to cancerocidal doses than previously. With the orthovoltage instruments, only patients with tumors less than 8 to 10 cm. in diameter are given definitive treatment This does not mean, however, that the same proportion of patients who were salvageable from the 8- to 10-cm. group with 250 kv. are also salvaged by supervoltage used on larger tumors. If this were true we would have a very appreciable increase in the over-all cure rate. Biologically, as the tumor gets older and larger the chances of metastasis increase. Therefore, although the multi-million voltage units can deliver cancerocidal doses to considerably larger tumor volumes, possible long-term survival is limited to the number which are truly localized to the volume of tissue under treatment. Nevertheless, although the percentage increase in "cures" is small, supervoltage results in infinitely better palliation since this is directly proportional to the dose absorbed In addition, this palliation is obtained with minimal side effects of radiation A case in point is the report made by Fletcher (20) who compared the treatment of supervoltage with 250-kv x-rays on carcinoma of the base of the tongue. At one year the survival with supervoltage was 80 per cent while that with 250 kv. was only 20 per cent; however, at two years the survival for both groups was the same, namely 10 per cent. In this instance, the total patient-months of survival are greatly increased by supervoltage therapy.

The second important accomplishment is that large tumor volumes, accepted for palliation only, are better managed because higher, although less than cancerocidal, doses can be comfortably delivered. Very large volumes, previously rejected even for palliative therapy, can now be included for treatment with supervoltage therapy. High-energy radiations can be used in the re-treatment of tumors through areas previously treated to skin tolerance by orthovoltage beams (49) Hence, although the long-term survival of all patients with cancer has not been dramatically increased, the total number of patient-months has been greatly augmented.

The possibility of bone necrosis is reduced with supervoltage therapy because the energy absorbed in bone, fat, and muscle is approximately the same for a given exposure However, since considerably greater quantities of radiation are delivered to the tumor, the absolute absorption in bone can still rise to excessive levels.

Personal observation of the betatron leads me to the conclusion that a greater number of patients may tolerate a relatively larger amount of irradiation, but it is still impossible to determine at the outset which patients can be so treated A greater number of delayed reactions has also been observed in patients who have minimal reactions in the deep structures during the course of treatment. These two observations compel one to proceed with great caution in pushing the dose of supervoltage radiation significantly higher than that known to be tolerated in routine deep therapy (13).

Special mention should be made of the use of the electron beam (2, 32, 45, 61, 62). The depth of the effective penetration of electrons is dependent on voltage. A rule of thumb is that the therapeutic range in centimeters below the skin surface is equal to one-third of the Mev. Hence, one can treat lesions to a depth of 0.6 cm. with 2-Mev electrons and to a depth of 8 cm with 24-Mev electrons. Beyond this depth the energy absorbed drops off rapidly. In the case of x-ray beams, the dosage falls off beyond the 100 per cent level more or less exponentially as it passes through tissue Using an electron beam, one can treat the internal mammary chain by a direct port and not expect to get a significant dose in the lungs, thus eliminating the problem of radiation fibrosis (27, 33, 45, 61, 62).

Despite numerous clinical reports which are present in the literature since 1950, only impressions about the clinical indications and expected benefits can be drawn. The difficulty arises because no single institution has sufficient cases from which statistically significant material can be drawn However, as one collects individual reports an unusual unanimity of feeling develops. One gains the impression that supervoltage therapy is particularly useful in treatment of cancers of the brain; head and neck, especially maxillary antrum, hypopharynx and base of tongue; tumors of the lung, cancer of the breast; cancer of the urinary bladder; and cancer of the rectum

SPECIALTY SITES

Intracranial Tumors

Although tolerance of the normal brain to radiation is not high, it is not uncommon to experience difficulty in treating tumors of this structure with 250 kv. x-rays since relatively large, eccentric volumes must be irradiated Supervoltage provides a better distribution of energy within the tumor, minimal irradiation to the surrounding normal brain, and a faster and better regrowth of hair. Medulloblastoma, characterized by repeated recurrences and relatively long survivals, can be treated through the same portal area repeatedly without skin limitation Reports are available by Blomfield (3) and Arnold *et al.* (1), the latter's account indicating that 11 of 21 patients with malignant tumors survived over 12 months.

The use of this energy level for treating metastatic carcinomas of the brain is encouraging enough to be considered worth-while in a reasonable percentage of patients

Head and Neck

Despite the relatively small diameters and comparatively superficial location of tumors in the head and neck, supervoltage radiation therapy is particularly valuable in this region. Although multiple, small port, beam-directed therapy can deliver desired cancerocidal doses in this area with

orthovoltage, execution of the plan is often impractical. Supervoltage is *infinitely* more simple and, in an area where cosmetics are particularly important, it is appreciated by the patient. Smith (54) reports 11 of 13 laryngeal cancers with primary regressions. Subglottic extension was present in six, of which five were controlled The maxillary antrum, one of the most difficult areas *to which ionizing radiation can be applied, is excellently* managed by supervoltage irradiation using two portals at right angles to each other with compensating wedge filters (7, 57). The latter provides for homogeneity of dosage throughout the tumor-bearing volume. Schulz (53) is not impressed with the increase in effectiveness of supervoltage therapy over orthovoltage but does recognize its simplicity and decrease in untoward reactions. He reports 24 cases treated with 5000 r by supervoltage and 98 cases treated with similar dosage with 250 kv. Over a five-year period he reports 20 per cent and 18 per cent surviving, respectively. Grouping all head and neck tumors together, Fletcher (17) reports a 90 per cent regression rate, which is much higher than has been experienced with 250 kv Smith (54) points out excellent initial responses in 24 to 48 months in lesions in the floor of the mouth, and notes that five of seven *survivals had invasion of the bone at the time of treatment.* Two of these, subsequently requiring resection of the mandible for necrosis, failed to reveal tumor growth in the resected specimen

In the case of the betatron or in electron beam therapy, a simple homolateral port can be used for treatment of tonsillar lesions. Tapley *et al.* (55) failed to show significant differences in survivals when patients treated on the betatron were compared with those treated with the 250 kv. unit In this particular lesion this group found the tumor response to be independent of dosage Lesions in the head and neck region are discussed by *numerous authors (3, 7, 11, 17, 21, 33, 38, 50, 62)*

It has always seemed a paradox that radiotherapy controls primary lesions in the head and neck region, yet fails in treatment of metastatic cervical nodes. Radical resection is the best choice when operable; however, where the patients are clinically inoperable, supervoltage x-ray beams do accomplish more than orthovoltage has in the past. Yet, what it does accomplish is less than ideal The electron beam is particularly suited for the management of inoperable metastatic cervical lymph nodes A cancerocidal dose can be delivered to the area of interest without appreciable effect on the deep structures which limits the use of the *high-energy x-ray* beam

THORAX

The long-term survival of patients with carcinoma of the esophagus treated by supervoltage is not significantly different from the long-term results with orthovoltage As in other areas, the biology of the tumor, namely, early metastases, destroys the patient However, in a tumor of limited radiosensitivity, better palliation is obtained and a higher percentage

TABLE I

CARCINOMA OF THE LUNG

Collected Series Treated by Supervoltage Radiation

I Author	II Number of Cases	III Dose/Time	IV Number Surviving	V Months Survival (Cases column IV)
Watson (64)	193 42 supervoltage 151 250 kv.	55–7500 r/18–25 days	5 5	20 12
Haas *et al.* (34)	190 47 betatron 143 250 kv.	32–7500 r/26–36 days 19–4500 r/26–36 days	10 10	12 12
Morrison & Deeley (48)	199	4500 r/4 weeks	63	12
Smith (54)	145 (moderately extensive and extensive)		5	24–51
Guttmann (29, 31)	144	5–6000 r/5–6 weeks	44	12
Kutz (44)	173	5–7000 r	12	24

of disappearance of the primary lesion is effected by supervoltage therapy simply because higher doses can be consistently delivered. Fletcher (17) prefers not to accept patients with esophageal or pulmonary cancers because of their eventual poor prognosis. Smith (54) reports 4 of 15 patients alive after four years, one of whom had a lesion in the lower third of the esophagus which is considered to be an unfavorable site for radiotherapy.

Like the esophagus, the lung (Table I) is a particularly discouraging lesion to treat, again because of the natural history of the disease. However, Haas *et al.* (34) report 10 of 47 cases treated on the betatron survived an average of 19.6 months as compared with the usual 6-months survival of orthovoltage-treated patients He is supported by others. Watson (64) points to better palliation and a better survival rate for epidermoid carcinoma as compared with that of adenocarcinoma of the lung He has the impression that the combination of nitrogen mustard and supervoltage therapy may well give significantly better results than radiation alone

CERVIX

Cervical cancer in stages 1, 2, and 3, when managed by ionizing radiation, is primarily treated by the local application of radium In most clinics, external radiation is applied to supplement the dose to the nodes in the lateral pelvic wall However, the amount of external radiation so applied is usually of modest amount (2500 to 4000 r in three to five weeks) and satisfactory dosage can be obtained by orthovoltage. The use of supervoltage, however, is infinitely more satisfactory from the patient's stand-

point because of the lack of radiation sickness and skin reaction in a sensitive area (30). Most patients gain weight during a course of external radiotherapy to the pelvis when treated by high-energy machines.

In the advanced cases (stage 4), however, it is necessary to treat the entire pelvis by external radiation therapy and almost always without the use of radium. Here doses of 5000 r over a five-week period are readily accomplished by supervoltage therapy. This large volume cannot be given this quantity of radiation by orthovoltage treatments. Again, few untoward effects are experienced. Rotation therapy, although not necessary, is particularly useful in this situation (60).

BLADDER

This is perhaps the area in which the greatest number of authors are enthusiastic about the use of supervoltage radiation (Table II). In a 1938 report, Dresser & Spencer (15) found treatment of the bladder with a 1.2-Mev Van de Graaff generator to be the most interesting area when compared with orthovoltage therapy. Similar encouraging impressions are obtained by others (7, 17, 37, 38). Cuccia *et al.* (14) report 100 cases treated on either betatron or cobalt[60] units. Although both instruments are satisfactory, the dose distribution through the tumor volume is somewhat better on the betatron. As seen in Table II, amelioration is possible not only in those patients with tumors limited to the bladder but also in those patients whose disease extends to the pelvic wall. They have found that older

TABLE II

CARCINOMA OF THE URINARY BLADDER

Collected Series Treated by Supervoltage Radiation

I Author	II Number of Cases	III Dose/Time	IV Number Surviving	V Months Survival (Cases column IV)
Busby (9)	58 24 radical 34 palliative	5–6000 r/3–6 weeks 3–4000 r/3–4 weeks	13 7	60 60
Burkell & Watson (7)	21	55–7200 r/19–38 days	13	
Plenk *et al* (51)	23	5–7000 r/6–9 weeks	11	30
Cuccia *et al* (14)	100 64 radical	55–6000 r/5 weeks	16 24 11	24 12 6
	31 palliative	35–4500 r/3–4 weeks	1 8 12	24 12 6

women do not tolerate the treatments well They also found that low-grade, low-stage lesions tend to recur despite whole bladder irradiation, and concluded that lesions in this superficial group are best handled by transurethral surgery.

Cantril & Buschke (11) report 9 of 61 patients alive after a five-year period. Smith (54), in his five-year summary on cobalt[60] beam therapy, reports four patients well for three to four years. He recommends supervoltage treatment for patients with: (*a*) recurrent papilloma; (*b*) bulky cancers beyond segmental resection; (*c*) large proliferating lesions involving trigone or ureteral orifices; (*d*) recurrence after fulgeration otherwise requiring cystectomy; and (*e*) those who refuse cystectomy. He points out that 58 per cent of those cases unsuitable for cystectomy because of local extent receive sufficient palliation to warrant supervoltage therapy Our own observations agree with others who find that patients with severe cystitis presenting for treatment have a decreased tolerance for this type of treatment. Perhaps as many as 10 per cent of patients treated radically will develop a contracted bladder. In patients with pain as a major symptom in advanced disease with small capacity and extra-cystic extension, consideration should be given to urinary diversion before the institution of treatment.

Rectum

Notable results can be obtained by the use of supervoltage therapy in the treatment of inoperable and recurrent rectal cancer (Table III). Phillips (50), in his early excellent monograph on supervoltage x-ray therapy, drew attention to this site. He reports 17 of 45 cases with dis-

TABLE III

CARCINOMA OF THE RECTUM

Collected Series Treated by Supervoltage Radiation

I Author	II Number of Cases	III Dose/Time	IV Number Surviving	V Months Survival (Cases column IV)
Phillips (50)	45	4–6000 r/4–6 weeks	17 (complete regressions)	
Blomfield (3)	25		3	24
Williams & Horowitz (65)	165 primary lesions	6000 r/6–8 weeks	9	60*
Williams *et al* (50)	82 recurrent after surgery	6000 r/6 weeks	1	60

* See text for interval survival data.

point because of the lack of radiation sickness and skin reaction in a sensitive area (30). Most patients gain weight during a course of external radiotherapy to the pelvis when treated by high-energy machines.

In the advanced cases (stage 4), however, it is necessary to treat the entire pelvis by external radiation therapy and almost always without the use of radium. Here doses of 5000 r over a five-week period are readily accomplished by supervoltage therapy. This large volume cannot be given this quantity of radiation by orthovoltage treatments. Again, few untoward effects are experienced. Rotation therapy, although not necessary, is particularly useful in this situation (60).

Bladder

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TABLE II

Carcinoma of the Urinary Bladder

Collected Series Treated by Supervoltage Radiation

I Author	II Number of Cases	III Dose/Time	IV Number Surviving	V Months Survival (Cases column IV)
Busby (9)	58 24 radical 34 palliative	5–6000 r/3–6 weeks 3–4000 r/3–4 weeks	13 7	60 60
Burkell & Watson (7)	21	55–7200 r/19–38 days	13	
Plenk *et al* (51)	23	5–7000 r/6–9 weeks	11	30
Cuccia *et al* (14)	100 64 radical	55–6000 r/5 weeks	16 24 11	24 12 6
	31 palliative	35–4500 r/3–4 weeks	1 8 12	24 12 6

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appearance of the lesion. More recently Williams and co-workers (65, 66) reported on 165 primary cases treated over a 12-year period, and on 82 patients who suffered recurrence following surgical removal of the rectum. Thirty-three per cent survived 2 years; 14 per cent for 3 years; and 5 per cent for 5 years in the 165 primary cases, 82 per cent of whom were accepted for therapy with tumor beyond the rectum. Patients so treated have a reasonable chance for complete or partial relief of symptoms, the possibility of avoiding colostomy and a five per cent chance of a five-year "cure."

These results in a tumor of relatively low radiosensitivity can be used as a model for one's attitude about resistant lesions elsewhere (28). Although startling results are not available, the use of supervoltage therapy for the palliation of any localized gastrointestinal carcinoma too extensive for surgery should be considered. A willingness to undertake the treatment of such cases, which would not be considered for orthovoltage, is possible because one can administer the necessary high doses by supervoltage radiation therapy without significant untoward effects during treatment.

SUMMARY

Without sufficient follow-up data, especially those suitable for statistical comparison with results of 250-kv. radiation therapy, one is not in a position to predict an increase in long-term survivals of patients treated with supervoltage irradiation. In fact, as pointed out previously, those few available papers reporting on five-year survival results are unimpressive. If the cumulative survival is considered as well as the well-being of the patient both during and after treatment, a significant contribution has been made by the adoption of high-energy generators.

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HEMATOLOGY: CONTROL OF RED CELL PRODUCTION[1]

By Allan J. Erslev, M.D

The Cardeza Foundation, Jefferson Medical College of Philadelphia, Philadelphia, Pennsylvania

In a monumental work on the physiologic effect of air pressure published in 1878 (1), Bert predicted that the low oxygen pressure found at high altitudes would result in an adaptive increase in the oxygen-carrying capacity of blood. This remarkable prediction was rapidly confirmed and it led eventually to the now generally accepted hypothesis that the rate of red cell production is adjusted to the tissue tension of oxygen Recent studies have clarified many of the operational features of the mechanism responsible for this adjustment. However, it is still not completely understood and its elucidation remains a fascinating and frustrating challenge. No attempt will be made here to give a comprehensive review of the large and somewhat confusing literature generated by this challenge, especially since several excellent and thorough reviews are available (2 to 5). Instead, this article will deal with experimental observations from the literature in a selective, critical, and possibly biased manner in order to try to delineate the physiologic feedback circuit which controls red cell production

The combined mass of mature and immature red blood cells constitutes a large organ, the erythron, designed primarily for the purpose of bringing oxygen from the lungs to the tissues. As is true of other organs, its size is carefully controlled with senescent and dying cells being replaced continuously and accurately by new cells. This fine adjustment of cellular proliferation to the size of the organ not only maintains the steady-state size, but operates when a large part of the organ is removed as in bleeding anemias or when the organ size is artificially increased as in transfusion polycythemia. Both conditions will lead to compensatory changes in the rate of cellular proliferation until normal size of the organ has been restored. The preservation of a constant size obviously demands the presence of a regulating mechanism sensitive to even minor fluctuations in the size and functional capacity of the red cell mass, and capable of inducing compensatory changes in the rate of red cell production.

Since the red cell mass is designed almost exclusively for the purpose

[1] The survey of the literature pertaining to this review was concluded in August, 1959.

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ing between the erythropoietic tissue and the other tissues in the body The pathway of this feedback mechanism is as follows: The rate of red cell production determines the size of the red cell mass, the red cell mass determines the hemoglobin concentration, the hemoglobin concentration determines the degree of tissue oxygenation, and the degree of tissue oxygenation determines the rate of red cell production.

Although there is general acceptance of the importance of this feedback mechanism, the possibility that other regulatory mechanisms may exist must be kept in mind It is not uncommon in hereditary spherocytosis to find increased erythropoietic activity despite a normal hemoglobin concentration (11, 12) This so-called "compensated hemolytic syndrome" has also been reported in dogs with plastic ball valve prosthesis of the Hufnagel type (13), and in patients with chronic primaquine-induced destruction of red cells (14). Such a syndrome cannot readily be explained if the normal hemoglobin concentration indicates a normal degree of tissue oxygenation. However, splenectomy and cessation of the hemolytic process in hereditary spherocytosis is always followed by a rise in hemoglobin concentration even when the presplenectomy hemoglobin concentration was within normal limits (11, 12). It is possible that the spherical shape of the red cells leads to a slower oxygen exchange in the capillaries and that the normal hemoglobin concentration observed in the "compensated hemolytic syndrome" represents a level inadequate for normal tissue oxygenation Recently, Brecher & Stohlman (5) have suggested that red cell production is controlled both by the tissue tension of oxygen and by an inhibitor released from old senile red cells This inhibitor of the erythropoietic tissue is supposed to be formed in the red cells during the aging process of the cell An increase in red cell production following blood loss or hemolysis would, according to their theory, be caused partly by the removal of inhibitor-containing red cells, and a decrease in red cell production would follow transfusion of inhibitor-containing red cells This would provide a mechanism by which red cell production is governed by the size of the red cell mass quite apart from its functional capacity. However, no experimental evidence for the existence of such an inhibitor has been provided and, as mentioned previously, the bone marrow in hypertransfused animals does not appear to be inhibited at all (7) Furthermore, an expansion of the red cell mass without a concomitant increase in the hemoglobin concentration, as observed in patients with large, blood-engorged spleens, is not associated with a suppressed rate of red cell production Actually, most, if not all, clinical and experimental observations on the physiologic control of red cell production are compatible with the sole existence of one regulatory mechanism based on the oxygen-carrying capacity of the red cell mass.

Since the pathway of this regulatory mechanism is tied in with many other physiologic systems it may be advantageous to consider it step by step.

oxygen in the capillaries for this diffusion, only a fraction of the total oxygen carried to the tissues is extracted. If the red cell mass decreases, the hemoglobin concentration and the oxygen-carrying capacity are diminished and a greater fraction of the transported oxygen has to be extracted in the tissues in order to meet the cellular demand. The result is that the arteriovenous oxygen difference increases, the mean capillary oxygen tension falls and cells situated at a distance from the capillaries suffer from hypoxia. On the other hand, if the red cell mass increases, the hemoglobin concentration and the oxygen-carrying capacity rise and the delivery of a sufficient amount of oxygen to the tissues can be accomplished with only a small difference between arterial and venous oxygen content The result will be a high mean capillary oxygen pressure with tissue cells being exposed to oxygen under abnormally high tension. In other words, changes in the red cell mass will result in tissue hypoxia or hyperoxia. Consequently, it seems most likely that the mechanism which controls the rate of red cell production and the size of the red cell mass is triggered by the metabolic effect of these changes in the tissue tension of oxygen This old and venerable hypothesis is supported overwhelmingly by observations of the hematologic effect of tissue hypoxia or hyperoxia induced by other mechanisms than a change in the red cell mass A decreased supply of oxygen to the tissues, as at high altitudes, or in patients with pulmonary disorders or right-to-left vascular shunt, will result in a low mean capillary oxygen tension, cellular hypoxia, and an accelerated rate of red cell production (6). An increased cellular demand for oxygen as after the administration of drugs like dinitrophenol (7) or triiodothyronine (8) results in functional tissue hypoxia and an increased erythropoietic activity An increased supply of oxygen to the tissues, as induced by breathing air with a high oxygen tension, will result in a high mean capillary oxygen tension, cellular hyperoxia, and a decreased rate of red cell production (9). Finally, a decreased cellular demand for oxygen as observed after hypophysectomy and possibly after starvation (8) will lead to functional tissue hyperoxia and is associated with a decreased rate of red cell production In short, clinical and experimental observations show that tissue hypoxia stimulates red cell production and tissue hyperoxia suppresses red cell production Rather than assuming the separate existence of a stimulator and a suppressor, it has been proposed that normally oxygenated tissue will provide a certain stimulus to the erythropoietic tissue; hypoxia will increase the intensity of this stimulus and hyperoxia will decrease it (10). Recent studies have supported this idea by demonstrating that erythropoietic tissue "suppressed" by hyperoxia is not inhibited at all but is actually very sensitive to the influence of an erythropoietic stimulus (7)

All these observations and considerations lead us finally to the hypothesis that the size of the erythron is controlled by a feedback mechanism operat-

adequate for normal cellular development, the rate of red cell production is determined solely by the rate of stem cell differentiation while the rate of subsequent multiplication and maturation is constant

Under steady-state conditions the rate of red cell production will support a daily release of red cells from the bone marrow equalling the daily destruction of red cells Since the normal daily destruction of red cells amounts to 1/120 of the red cell mass which, in an adult male weighing 70 kilograms, is approximately 2500 ml, the daily output of red cells must be around 20 ml. In response to appropriate physiologic stimuli the erythropoietic tissue has the capacity to decrease this output to almost zero (25) or to increase it six- or sevenfold (26) Only a small fraction of this wide functional range is utilized when the bone marrow has to compensate for tissue hypoxia as long as the red cell lifespan is normal. At high altitudes or in cyanotic heart diseases a mere doubling of the rate of red cell production will result in pronounced polycythemia with a red cell mass of twice normal. It is understandable, therefore, that it is often quite difficult to demonstrate increase in red cell production in mild to moderate polycythemia On the other hand, when the rate of red cell production is increased in order to compensate for a hemolytic anemia where the lifespan of the red cells has been reduced to a small fraction of normal, the full compensatory capability of the bone marrow may have to be mobilized with obvious morphologic and functional changes in the erythropoietic tissue.

A pathologic impairment in the rate of red cell production, as in anemias attributed to nutritional deficiencies or to defects in the structure of the nucleated red cells, will cause a decrease in the red cell mass and a restriction in the capacity of the bone marrow to compensate for this decrease. Similarly, a pathologic increase in the rate of red cell production as in polycythemia vera will lead to an increase in the red cell mass without the bone marrow being able to compensate for this increase. Consequently, when the metabolic environment is adequate for the maturation and multiplication of red cells the rate of red cell production can vary over a wide range, giving the red cell mass both stability and flexibility. However, when the erythropoietic tissue is impaired the range of red cell production is narrowed and the red cell mass can no longer adapt its size to the environment or compensate for pathologic gains or losses.

From Red Cell Mass to Hemoglobin Concentration

Since the oxygen supply to a specific tissue depends on the amount of oxygen-containing hemoglobin in the blood perfusing the tissue, the functional importance of the red cell mass is determined by the hemoglobin concentration it can maintain in circulating blood [illegible]

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From Red Cell Production to Red Cell Mass

The size of the red cell mass is determined by the rate of red cell production and by the mean lifespan of the circulating red cells. The lifespan may vary from a few days in severe hemolytic anemias to a normal of 120 days and has been reported to be even longer in myxedematous patients (15). However, it is not a physiologic variable and the bone marrow alone is responsible for maintaining the red cell mass at a size optimal for the fulfillment of its functional objectives

The red cells are produced in the erythropoietic tissue by differentiation of multipotential stem cells into pronormoblasts capable of multiplication and hemoglobin synthesis (16). Following the differentiation of a stem cell into a pronormoblast, multiplication proceeds through mitotic division until 2 to 3 days later when the nucleus has become pyknotic and incapable of further deoxyribonucleic acid synthesis. Cytoplasmic hemoglobin synthesis occurs simultaneously, associated with a gradual decrease in ribonucleic acid until only a few brilliant cresyl blue-staining remnants are left. At this point the pyknotic nucleus is extruded, transforming the nucleated normoblast into a reticulocyte. During the next 2 to 4 days, while the final synthesis of hemoglobin takes place, the reticulocyte loses some of the stickiness which has helped to keep it stationary in the bone marrow (17) and it is flushed out into the circulation. After approximately 120 days the protective enzymes wear out and the cell is destroyed with its component parts either reutilized or excreted.

The rate of red cell production could be controlled by the rate at which stem cells are differentiated into pronormoblasts or by the rate of multiplication of these pronormoblasts, or by both. However, recent studies have shown that the rate of mitotic division of individual nucleated red cells in erythropoietic tissue "suppressed" by hyperoxia is the same as in erythropoietic tissue stimulated by anemic hypoxia (16). Furthermore, Steele (18), Huff and co-workers (19), Schwartz (20), and Cooperberg & Singer (21), found the same ratio between pronormoblasts and normoblasts whether the rate of red cell production was suppressed or accelerated In addition, Alpen & Cranmore (22) have demonstrated that the intermitotic period of nucleated red cells in anemic dogs is the same as in normal dogs, and Kriss and co-workers (23) have shown that acute transfusion polycythemia does not interfere with the metabolism of already formed nucleated red cells It is, of course, well known that the time spent by the maturing cells in the bone marrow engaged in rapid red cell production is shortened with early reticulocyte and even nucleated red cells appearing in the blood stream. However, this premature release may merely be caused by an increased intramedullary growth pressure (24), and there is no evidence that the time required from differentiation to final maturation is shortened. Consequently, it has been concluded that as long as the metabolic environment is

where between the arterial and the venous oxygen tension (31). The arterial oxygen tension is the same throughout the body and is determined by the oxygen tension in the atmosphere and by the functional and anatomic integrity of the lungs and heart The venous oxygen tension, on the other hand, varies from tissue to tissue and is determined by the amount of oxygen delivered to the tissue and by the amount consumed by the tissue. The amount delivered depends on a number of factors, the oxygen tension in atmospheric air, the pulmonary function, the hemoglobin concentration, the cardiac output and the vascular distribution of circulating blood among the individual tissues From this amount of oxygen a certain fraction is extracted and consumed in the tissue, and the oxygen tension in the capillary blood and in the venous blood leaving the tissue depends on the amount of oxygen left over and the shape of the oxygen dissociation curve Consequently, many factors in addition to the hemoglobin concentration are involved in the establishment of a pressure gradient along which the oxygen molecules can flow from the atmospheric air to the cells. A change in any one of the factors will result in a change in the slope of the oxygen gradient and the cellular supply of oxygen will exceed or fall short of the demand The resulting change in the cellular metabolism will, in some way, mobilize compensatory mechanisms until the steady-state has been restored In the individual tissue there may be an increase or a decrease in the vascularity with a change in the distance between the cells and the nearest capillary and a change in the amount of blood which perfuses the tissue. Furthermore, the mean capillary oxygen tension may be altered by appropriate changes in the oxygen dissociation curve, in the pulmonary vital capacity, in the cardiac output and in the rate of red cell production Of all these mechanisms a change in the number of active tissue capillaries appears to be the compensatory device most responsive to minor fluctuation in the degree of tissue oxygenation However, an increase or a decrease in the rate of red cell production seems to be the preferred way in which the tissues attain compensation for major and for prolonged changes in tissue oxygenation Since a change in the red cell production only slowly results in an effective change in the oxygen-carrying capacity of blood, temporary pulmonary and cardiovascular adjustments have to be called into action to protect the tissues until the steady-state has been restored

Under normal conditions a steady-state is achieved when the red cell production proceeds at a rate which will maintain a circulating red cell mass of approximately 35 ml per kg of body weight and a hemoglobin concentration of 14 5 gm per 100 ml of whole blood Why this specific red cell mass and hemoglobin concentration are "chosen as normal" is not known. Theoretically, an equilibrium between oxygen supply and oxygen demand can be reached at any hemoglobin concentration as long as pulmonary and cardiovascular activities are properly adjusted. One reason may

to maintain a hemoglobin concentration optimal for oxygen transport, the rate of red cell production and the red cell mass have to be adjusted to the size of the total blood volume If the blood volume changes, as in dehydration or in congestive failure (27), the hemoglobin concentration is altered temporarily but an immediate compensatory adjustment in the rate of red cell production will result in a change in the red cell mass and an eventual return of the hemoglobin concentration to normal. However, the rate of red cell production must, in addition, be adjusted to the effect that a change in blood volume has on cardiac output and the perfusion of tissues with blood. Such an adjustment can be observed in experimental dilution anemia (28) where, despite the anemia, the red cell production is reduced as a compensatory adjustment to the increase in cardiac output and the better perfusion of the tissues.

In most anemias and polycythemias the blood volume stays constant because even minor changes in blood volume may impair its essential function of "keeping the vascular system adequately distended in order to insure the maintenance of the venous return" (29). However, in severe anemia with a profound diminution in red cell mass the blood volume may contract in order to prevent the hemoglobin concentration from falling below a level compatible with life (30). Likewise, in severe polycythemia at high altitude or in patients with congenital cyanotic heart disease, the blood volume may expand, reducing the hemoglobin concentration and the hematocrit, presumably in order to keep the accelerated red cell production from clogging up the circulation with viscous blood. The separate, but, at certain points, integrated mechanisms which control red cell production and blood volume will determine the hemoglobin concentration and both mechanisms have to be accounted for in the evaluation of the functional significance of a change in hemoglobin concentration

From Hemoglobin Concentration to Tissue Oxygenation

The hemoglobin concentration alone will determine the amount of oxygen which can be carried per unit of circulating blood but the amount of oxygen which actually is made available to the cells for their oxidative metabolism depends on the integrated action of a number of tissue systems Oxygen molecules are picked up in the lungs from the atmospheric air, bound to the hemoglobin molecules and brought to the tissue capillaries Here they must be present under a pressure high enough to permit them to leave the capillary, diffuse through the tissue and reach even the most distant cell (6). In a highly vascular tissue with a rich capillary supply a fairly low mean capillary oxygen pressure will be sufficient to ensure all tissue cells of an adequate supply, while a poorly vascularized tissue has to depend on a high mean capillary oxygen tension. The mean capillary oxygen tension which determines the tissue perfusion pressure lies some-

of erythropoietic activity in all the long bones despite a much lower oxygen saturation of the blood perfusing the hind legs than the forelegs.

These studies, while making untenable the hypothesis that oxygen controls red cell production directly, have given solid experimental backing to an equally old, alternative hypothesis of the control of red cell production. In 1906, Carnot & Deflandre (44) postulated that the rate of red cell production is regulated indirectly by a humoral factor, produced somewhere outside the bone marrow in response to tissue hypoxia and carried to the bone marrow via the blood stream. During the next decades a number of investigators attempted to study this factor and describe its nature and properties [see reviews by Hirsjärvi (45) and by Grant & Root (2)] However, Gordon & Dubin in 1934 (46) and Feenders in 1936 (47) convincingly showed that serum in the small amounts employed by Carnot & Deflandre and by their many adherents had no measurable erythropoietic activity. A breathing spell followed, but the attractiveness of the hypothesis that red cell production is controlled by a humoral factor, plus the observation made by Reissmann, led to a re-examination of the possible erythropoietic activity of plasma from anemic animals. This time, however, plasma was employed in large quantities on successive days in order to minimize dilution of the factor in the recipient's own plasma. Utilizing this technique, Krumdieck (48), Gunther and coworkers (49), and Erslev (50) succeeded in demonstrating that plasma and serum from rabbits with bleeding anemia will induce a reticulocytosis in normal rabbits. Numerous studies have subsequently confirmed these findings, and have shown that serum or plasma from animals with blood-loss anemia or with hemolytic anemia, when infused into normal animals, will, in addition to a reticulocytosis, induce an increase in red cell count (50 to 52), hemoglobin concentration (51, 52), hematocrit (50, 51) number of nucleated red cells in the marrow (48, 50), radioactive iron clearance rate (53), and rate of red cell utilization of radioactive iron (54, 55). No definite effect has been observed on the rate of white cell and platelet production (50), or on the intestinal absorption of iron (56), and so far serum from anemic animals has not been shown to have any metabolic action on tissues other than the erythropoietic tissue.

The demonstration that plasma from anemic animals contains an erythropoietic principle, referred to variously as erythropoietic factor or erythropoietin, was quickly followed by studies showing a similar erythropoietic principle in plasma from animals exposed to low atmospheric oxygen tension (57) It was shown, furthermore, that there is a rough direct relationship between the degree of tissue hypoxia and the level of erythropoietic factor in plasma. The level of erythropoietic factor in the plasma from normal ani-

be that the viscosity and the red cell count of blood at this hemoglobin concentration permit a maximal flow of hemoglobin-containing red cells through a narrow tube (32). The many factors which influence the transport of oxygen to the tissues obviously make it difficult quantitatively to relate a given hemoglobin concentration to the degree of tissue oxygenation On the other hand, when the oxygenation of the tissue is altered there appears to be a regular and predictable change in the rate of red cell production and hemoglobin concentration.

From Tissue Oxygenation to Red Cell Production

The functional capacity of the erythropoietic tissues, like that of other oxygen-dependent tissues, is undoubtedly influenced by its supply of oxygen Since proliferative activity is stimulated under conditions in which the body's overall oxygen supply is insufficient to meet its oxygen demand, and is suppressed when the supply exceeds the demand, it has been assumed that a decrease in the oxygen supply to the normoblasts would result in metabolic processes leading to an accelerated proliferative activity while an excess of oxygen would suppress the rate of proliferation. Studies designed to test this hypothesis, first proposed by Miescher in 1893 (33), have not supported it although they have never ruled it out altogether. Thomas (34) found that the amount of hemin synthesized by a suspension of bone marrow is not increased by exposure to low oxygen tension, and Erslev & Hughes (35) found that the mitotic activity of nucleated red cells *in vitro* is suppressed rather than accelerated by a low oxygen tension. Rosin & Rachmilewitz (36) and Magnussen (37), working with bone marrow transplants rather than cellular suspensions, likewise failed to find a reciprocal relationship between oxygen tension and proliferative activity. Pathologic examination of bone marrow from extremities amputated because of arterial disease and tissue ischemia did not reveal any erythropoietic activity (38). However, this observation is hard to evaluate since the simultaneous reduction in temperature of the affected limbs may have reduced erythropoietic activity (39) and obscured a possible stimulating effect of the ischemic hypoxia. A major setback for the hypothesis that the oxygen tension in the bone marrow regulates erythropoietic activity directly was provided by Reissmann in 1950 (40). He demonstrated an accelerated erythropoietic activity in the bone marrow of the normal, well-oxygenated parabiotic partner of a rat exposed to low atmospheric oxygen tension. In 1954 Stohlman *et al.* (41) and in 1955 Schmid & Gilbertsen (42) provided more evidence against this hypothesis. They observed that patients with reverse flow through a patent ductus arteriosus, causing cyanosis and hypoxia in the lower half of the body, developed erythropoietic hyperplasia not only in the bone marrow of the hypoxic lower half of the body but in the upper half as well A similar observation was made by Eränkö & Karvonen in foetal sheep (43). They found the same degree

tion regarding erythropoietic factor has been obtained by means of this technique, but it is obviously crude and laborious and necessitates the use of large volumes of plasma. Innumerable variations on this bioassay theme have been tried out, but so far no completely reliable method has been designed. Different erythropoietic parameters have been followed, the recipients have been rendered more sensitive to the infused plasma, and the plasma has been deproteinized in order to make it possible to perform bioassay of plasma from one species in a recipient of another species.

The erythropoietic response is usually evaluated from reticulocyte counts or from the 20-hr. red cell utilization of radioactive iron, but red cell count, hemoglobin concentration, bone marrow examination, and serum iron turnover have been utilized as well. The reticulocyte response as evaluated from daily reticulocyte counts over a one-week period has long been known to give a good indication of change in erythropoietic activity. However, it is not a quantitative measurement since it depends both on rate of red cell production and rate of release of reticulated cells from the marrow. The 20-hr. utilization of radioactive iron is far easier but it is even less quantitative since it depends on the competitive utilization of iron between bone marrow and other tissues, the reticulocyte level in circulating blood and the rate of release of reticulocytes from the marrow. These factors all bear a qualitative but not quantitative relationship to the rate of red cell production (63). Despite these shortcomings, both tests have been quite useful in estimating the level of erythropoietic factor in an unknown plasma sample (64).

In order to obtain a clear-cut difference between the rates of red cell production before and after the infusion of plasma, animals with a low baseline level of erythropoietic factor and a slow rate of red cell production have been used as recipients. Transfusion polycythemia or exposure to high atmospheric oxygen tension has been utilized to induce such a reduction in erythropoietic activity, and it has been shown that these hyperoxic recipients are far more "sensitive" than normal animals to the effect of erythropoietic factor (65). Hypophysectomized and starved animals have similarly been utilized as recipients on the assumption that their low metabolic rates would result in a relative hyperoxia of the tissues and a low level of erythropoietic factor in their circulating bloods (65). Since whole plasma may provide hormonal or nutritional substances needed by these animals, an erythropoietic response to plasma is often hard to evaluate unless matched with carefully designed control experiments. Stohlman & Brecher (66) and Korst (67) have used recipients in whom the rate of red cell production has been suppressed by means of sublethal irradiation or nitrogen mustard. At the present time there is no good theoretical reason why these animals should be more sensitive to erythropoietic factor than normal animals. Both radiation (68) and nitrogen mustard (69) suppress erythropoietic activity without changing the level of circulating erythropoietic factor, and one would have

crease in the red cell utilization of radioactive iron (58). When tissue hypoxia is induced, erythropoietic activity can be demonstrated within a few hours In rabbits this activity increases gradually during the next 24 to 48 hr., after which it remains stationary as long as the tissue hypoxia is maintained If the hypoxic state is terminated abruptly the level of erythropoietic activity falls and becomes immeasurable within 3 to 6 hr. (59) A similar course takes place in rats, with the exception that erythropoietic activity reaches a peak value 24 hr. after the onset of tissue hypoxia and then decreases moderately (60). The highest level of activity is found in plasma from animals with severe phenylhydrazine-induced hemolytic anemia where the hemoglobin concentration may be as low as 3 gm per cent, and part of this hemoglobin even transformed into inert methemoglobin (61). A somewhat lower level of activity is found in anemia induced by bleeding where the hemoglobin concentration rarely dips below 5 gm per cent. In tissue hypoxia induced by exposure to low atmospheric oxygen, Stohlman & Brecher (62) have shown directly that the level of erythropoietic activity in plasma increases progressively as the oxygen tension decreases. The level of erythropoietic factor in plasma from animals with transfusion polycythemia or animals exposed to high atmospheric oxygen tension is low, but whether or not it is lower than in normal animals is impossible to assess directly with our present bioassay technique. However, studies carried out by Jacobson and co-workers have demonstrated that animals rendered hyperoxic by means of transfusion polycythemia will have a relatively greater response to the administration of a known amount of erythropoietic factor than will normal animals (7) This would suggest that the level of endogenously produced erythropoietic factor is lower in the hyperoxic than in the normal recipients In short, the level of erythropoietic factor reflects the tissue tension of oxygen quite accurately. Since, in addition, its appearance in the blood stream seems to precede increased bone marrow activity it appears to fulfill the requirements of a mediating "hormone," relaying information about the degree of tissue oxygenation to the bone marrow

It is still necessary to be cautious in the interpretation of these experimental data since the measurement of the level of erythropoietic factor in the blood stream is made by crude and semiquantitative bioassay methods The techniques employed are as numerous as the investigators and, although the above-mentioned observations have been confirmed repeatedly, other aspects of the production, metabolism, and character of the erythropoietic factor are still hotly disputed

The simplest and still most convincing method of demonstrating erythropoietic factor in plasma is to infuse the plasma into normal recipients of the same species and observe its erythropoietic effect Normal plasma is used as a control to give a semiquantitative evaluation of the amount of erythropoietic factor present in the unknown plasma sample. Most of the basic informa-

of boiled, partly-inactivated plasma. Actually, only plasma concentrated manyfold and obtained from severely anemic patients could be anticipated to yield an extract with erythropoietic activity. It seems indeed very improbable that boiled plasma from any polycythemic patient would show much residual activity, since the degree of erythropoietic activity in polycythemic patients as mentioned previously is probably lower than the degree of activity in even moderately anemic patients The final elucidation of the importance of the erythropoietic factor in human pathology must await the development of a better bioassay technique, but it does appear unlikely that physiologic principles operating in lower mammals should not be relevant for human beings as well.

In order to complete our feedback circuit, information is needed as to where in the body the erythropoietic factor is produced or released. It is tempting to assume that the erythropoietic factor behaves like a hormone and is produced in one specialized endocrine organ capable of responding to a low tissue tension of oxygen with the production of an erythropoietic factor. Since physiologic suppression of the activity of this hypothetical organ by means of hypertransfusion or exposure to oxygen will lead to the disappearance of all erythropoietic activity, removal of this organ must result in a complete cessation of red cell production. Many attempts have been made to localize the responsible organ in this manner, but so far no organ has been found to be absolutely essential for red cell production The removal of spleen, endocrine organs, thymus, or gastrointestinal tract has not abolished red cell production, and the administration of thorium dioxide (Thorotrast), nitrogen mustard, or x-radiation has not curtailed the production or release of erythropoietic factor. Removal of certain vital organs like the liver, central nervous system, heart, or lungs has not been possible. Extracts of these organs have been devoid of erythropoietic activity (77), but it is still possible that one of them may harbor the site of erythropoietic factor production Most attention has been paid to the kidneys because of the clinical observations that renal impairment is associated with a decrease in erythropoietic activity and renal tumors occasionally are associated with polycythemia In 1957, Jacobson and co-workers (78) reported that nephrectomy in rats is followed by an almost total disappearance of erythropoietic factor from the blood stream, and that these azotemic animals are unable to respond to anemia, hypoxia, or cobalt with the normal production or release of erythropoietic factor. Furthermore, ureter-ligated rats, equally azotemic, were reported to respond to tissue hypoxia in a normal manner. The obvious conclusion was that the kidney tissue must be responsible for the production or release of erythropoietic factor This conclusion has been supported by further experimental work carried out by Jacobson and co-workers (79), by Naets (80, 81), and by Osnes (82), but it has been challenged by other investigators. Erslev (83) found the same degree of

anticipated that they would have responded less rather than more to exogenous erythropoietic factor. However, the results obtained by utilizing such animals as recipients indicate that they are quite sensitive to the action of erythropoietic factor, a finding which remains unexplained.

One of the first attempts made to improve the basic bioassay technique was, of course, the utilization of a large donor of one species and a small recipient of another species. However, the infusion of whole plasma from one species to another has in most hands given unreliable results, either because of an immediate "toxic" effect or a delayed immunologic effect of the foreign plasma (61). In 1954, Borsook and co-workers (70) discovered that plasma from anemic rabbits could be boiled for a short period without losing its erythropoietic activity. This procedure removed the great majority of the plasma proteins and made it possible to perform bioassay of plasma from humans or large animals in small animals like rats or mice The technique was successfully used in a number of laboratories, but in other laboratories boiling was found to remove all erythropoietic activity and the technique was considered useless. Recent studies by Gurney (71), Stohlman *et al.* (72), and Borsook *et al.* (61) have largely solved this controversy by showing that a considerable amount but not all of the erythropoietic factor is destroyed by boiling. By careful concentration of the heat-stable supernatant, an active extract with little cross-species toxicity can be obtained

Utilizing these refinements in the biossay technique, attempt has been made to isolate and identify the erythropoietic factor and to assess its role in human physiopathology. Biochemical analyses carried out by Rambach and co-workers (73) and by Lowy and co-workers (74) indicate that erythropoietic factor in plasma is present in the muco-gluco-protein fraction which electrophoretically is located in the alpha$_1$—alpha$_2$ globulins "Yet from the similarity in yield and electrophoretic behavior of the corresponding inactive fraction from normal plasma it appears likely that the erythropoietic factor constitutes only a small portion of the present concentrates" (74).

Isolation of the erythropoietic factor in quantities large enough to be used in therapeutic trials has as yet not been accomplished. The slow progress in purification may be aggravating but it does not appear to be a great disadvantage since much more needs to be known of its relation to human anemias and polycythemias before it can be employed intelligently as a therapeutic agent. It has been reported that plasma from the majority of patients with anemias and from some patients with primary and secondary polycythemia is erythropoietically active [see review by Gordon (4)] The highest levels observed have been found in patients with aplastic anemia (75) and Cooley's anemia (76), in whom a substance with erythropoietic activity has been isolated both from plasma and urine These findings, however, have been capricious and inconsistent, as could be expected when one considers the crude bioassay technique employed in measuring the activity

that almost all clinical and experimental observations point towards a feedback circuit leading from the erythropoietic tissue through the red cell mass, the hemoglobin concentration, the tissue tension of oxygen, the erythropoietic factor, and back to the erythropoietic tissue again. The weakness of this theory lies in the fact that we do not know the organ or cellular system which releases the erythropoietic factor and feeds information about the tissue tension of oxygen to the bone marrow. Nevertheless, the proposed feedback control of red cell production appears to be our most challenging and most promising working hypothesis

erythropoietic suppression in nephrectomized and in ureter-ligated animals, and he found furthermore that although nephrectomy produces a profound suppression of the erythropoietic tissue, some degree of erythopoietic activity remains and can be observed as long as the animals are kept alive. His conclusion was consequently that azotemia rather than nephrectomy is responsible for the suppression of red cell production observed in renal disease or after nephrectomy. Further studies are obviously needed in order to resolve these experimental discrepancies, demonstrate the site or sites of the production of erythropoietic factor and provide us with the missing link in the feedback circuit governing red cell production.

The final tie-up with red cell production takes place in the bone marrow. As described previously, tissue hypoxia has been shown to control the rate of red cell production by regulating the rate of stem cell differentiation to nucleated red cells. If the working hypothesis is correct that the effect of tissue hypoxia is mediated by means of a circulating erythropoietic factor, the rate of stem cell differentation must be determined by the level of this factor in the blood perfusing the bone marrow. Much indirect evidence has already been presented to justify such a hypothesis. In addition, Althoff & Werner (85) have recently shown that the infusion of a plasma extract with high erythropoietic activity into normal recipients results initially in an increase in the most immature nucleated red cells and later in a generalized erythroid hyperplasia. This would indicate that the action of erythopoietic factor on the erythropoietic tissue is similar to if not identical with the action of tissue hypoxia on the bone marrow. So far, direct demonstration *in vitro* of the action of erythropoietic factor on bone marrow is not conclusive. It has been claimed that plasma from anemic animals containing erythropoietic factor will induce a normoblastic proliferation (86, 87), and an increased uptake of radioactive iron in bone marrow suspensions (88). However, most of these suspensions have probably not contained any of the all important stem cells in a viable state, and Erslev & Hughes (35) failed completely to find any difference in the uptake of radioactive iron of bone marrow suspended in normal serum and in anemic serum. Despite all these obvious experimental gaps, enough evidence exists to justify the assumption that erythropoietic activity depends on the level of an erythropoietic factor in the blood stream and that this level in turn depends on the tissue tension of oxygen.

Conclusion

It is generally accepted that the rate of red cell production is geared towards maintaining in the circulating blood a hemoglobin concentration optimal for the transfer of oxygen from the lungs to the tissue cells. In order to maintain or approximate such a hemoglobin concentration a sensitive feedback mechanism must exist between the erythropoietic tissue and one or many oxygen-consuming tissues. This review has attempted to show

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THE MEGALOBLASTIC ANEMIAS[1,2]

By D L. Mollin, M.D.
Department of Hematology,
Postgraduate Medical School of London, London, England

Megaloblastic anemia most frequently occurs in patients who are unable to absorb normal amounts of B_{12} or folic acid but it is also found in a variety of other conditions in which the pathogenesis of the anemia is more obscure. For example, although MA may occur in patients whose diet is

caused by cirrhosis (9, 10), or with kwashiorkor (11). The importance of these associated conditions is emphasized by the fact that MA may occur in some of them even in the absence of dietary deficiency or of intestinal malabsorption Megaloblastic anemia of pregnancy in the temperate zone, for example, frequently occurs in patients who maintain a good diet and whose intestinal function is normal (1, 12). A similar state is described in patients with MA associated with hemolytic anemia (3, 13 to 15) or myelofibrosis [(16, 17); and see (18)] In these patients the anemia probably develops because the tissue requirements for hemopoietic factors exceed the normal dietary intake In others, however, the megaloblastic change appears to arise from interference with the action of FA as, for instance, in patients taking anticonvulsant drugs (19, 20) or excessive doses of the malarial suppressant, pyrimethamine (21, 22) A similar inhibitory mechanism may explain the MA of tropical sprue (23) and may possibly be the cause of the megaloblastic change which is not uncommon in the refractory or partially refractory "sideroblastic" anemia that occurs in some patients with hemosiderosis and hemochromatosis [(24); and see (25, 26)].

In all of these conditions normoblastic hemopoiesis is restored if the patients are treated with B_{12} or FA. Megaloblastic change may, however, sometimes persist after treatment. Thus, it has recently been reported in a child with a defect in pyrimidine synthesis associated with the excretion of large amounts of orotic acid (27), and the megaloblastic change so frequent in Di Guglielmo's disease is invariably resistant to treatment (28).

The recent literature which is directly or indirectly concerned with all

[1] The survey of the literature pertaining to this review was concluded in July, 1959

[2] The following abbreviations will be used ATP (adenosinetriphosphate); CF (citrovorum factor); DNA (deoxyribonucleic acid); EDTA (ethylenediaminetetraacetic acid); IF (intrinsic factor); MA (megaloblastic anemia); PA (Addisonian pernicious anemia); RNA (ribonucleic acid); FA (folic acid); B_{12} (vitamin B_{12}).

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falls before the patients become anemic, the method has proved particularly useful for the diagnosis of B_{12} deficiency in (*a*) non-anemic or mildly anemic patients with PA who present with achlorhydria, macrocytosis, peripheral neuritis, or combined system disease (32, 44 to 46); (*b*) non-anemic subjects taking a strict vegetarian diet (47); (*c*) non-anemic patients with Crohn's disease (42); (*d*) patients who also suffer from deficiency of FA (48) or iron (4, 32). The frequency of iron deficiency in patients after gastrectomy and of FA deficiency in idiopathic steatorrhoea (adult coeliac disease) makes the B_{12} assay the only satisfactory diagnostic method for B_{12} deficiency in these conditions

Several test organisms may be used for these assays [see (40)]. The *Lactobacillus leichmannii 313* assay is a relatively simple, but time-consuming procedure which requires an incubation period of 48 to 72 hr. The *Euglena* assay is simpler and easier to carry out but requires more elaborate apparatus and needs a five-day incubation period. It is, however, the most sensitive and accurate assay available, particularly if the *z* strain of *Euglena gracilis* is used (49).

Serum B_{12} levels in uncomplicated B_{12} deficiency.—Using these assays, levels in normal subjects range from about 140 µµg. per ml. to 900 µµg. per ml and in untreated PA from less than 10 to about 100 µµg. per ml, usually being less than 80 µµg. per ml. (40, 43). An advantage of the *Euglena* assay is that very low levels (less than 50 µµg. per ml) are invariably found in patients with combined system disease irrespective of the degree of the anemia (44). The low B_{12} levels in PA reflect depleted tissue reserves for there is very little B_{12} in the tissues of untreated patients (50, 51)

Subnormal concentrations, similar in distribution to those seen in PA, are found in almost all patients with MA or other signs of B_{12} deficiency attributable to (*a*) dietary deficiency of B_{12}; (*b*) partial or total gastrectomy; (*c*) anatomical lesions of the small intestine; and (*d*) fish tape worm anemia (40 to 43, 52, 53) With few exceptions, the patients in these groups respond as completely to treatment with small doses of B_{12} as do patients with PA.

Serum B_{12} levels in other megaloblastic anemias.—Subnormal serum B_{12} levels are also found in 30 to 50 per cent of patients with idiopathic steatorrhoea whether anemic or not (42, 43, 54), in a large proportion of patients with tropical megaloblastic anemia and tropical sprue (4 to 6, 40), and in about 10 per cent of patients with megaloblastic anemias associated with pregnancy and the use of anticonvulsant drugs (43, 44) However, although the concentrations in these patients may be subnormal, the levels are frequently higher than those found in untreated PA, levels of less than 50 µµg per ml. being rarely seen except in certain patients with tropical sprue and tropical MA [(40); and see (4 to 6)]. In most of the patients in these groups who have subnormal levels the concentrations range

of the problems related to the megaloblastic anemias is too large to be reviewed in the space available and I have restricted discussion to publications dealing with the diagnosis, investigation, and pathogenesis of these anemias.

Morphological Diagnosis

Megaloblastic and intermediate megaloblastic change.—Typical megaloblastic change is easily recognised and its significance is generally appreciated. However, there is confusion about the significance of the "intermediate" megabloblasts (29 to 31). Trowell's observation (29) that the erythroblasts in the marrow of patients with MA complicated by severe iron deficiency are intermediate in appearance between megaloblasts and normoblasts, has recently been confirmed (31 to 33). Furthermore, it has been stressed that the underlying megaloblastic change may be unrecognisable when complicated by iron deficiency until the patients are treated with iron (33). A similar alteration in the appearance of megaloblasts has been noted in other conditions, such as Mediterranean anemia, severe trauma, or sepsis (13, 31).

However, it is incorrect to conclude (31) that intermediate megaloblasts are confined to conditions in which there is actual or effective iron deficiency and that they are never seen in uncomplicated PA. Intermediate megaloblasts are, in fact, the red cell precursors of many of the erythrocytes in PA when the anemia is moderate or mild (30), and the presence of intermediate megaloblasts has frequently proved to be an invaluable aid in the diagnosis of early PA (34 to 36). Intermediate megaloblastic change is, in fact, the characteristic marrow reaction in uncomplicated B_{12} or FA deficiency when the anemia is mild and hemopoietic demands are slight On the other hand, the presence of intermediate megaloblastic change in severely anemic patients does, almost certainly, indicate that the B_{12} or FA deficiency is complicated by a condition causing defective hemoglobin synthesis.

Cytological changes in other tissue—Nuclear abnormalities similar to those seen in the hemopoietic cells have been described in exfoliated buccal or gastric cells in patients with PA and other megaloblastic anemias [see (37)] Small numbers of abnormal cells have also been found inconstantly in preparations from the upper respiratory tract, bronchial tree, and genitourinary tract (38). The gastric and buccal cell abnormalities are not specific for B_{12} or FA deficiency for they occur in other conditions in which there is gastric or lingual atrophy (37). However, the fact that these cell changes disappear rapidly in PA after treatment may sometimes be of diagnostic value (39).

Assessment of B_{12} Deficiency

Serum B_{12} assay—The value of the serum B_{12} assay for diagnosing B_{12} deficiency is well established (40 to 44). Because the serum B_{12} level

FA and its known derivatives are absent On the other hand, others claim to have found material active for *Streptococcus faecalis* in the blood and plasma of normal subjects (66, 67). This material is absent from the blood of patients requiring treatment with FA and also from the blood of many patients with untreated PA (66).

Recently a modification of the *L casei* method for measuring serum "folic acid" activity in untreated patients has been described which gives results parallel to the hematological responses to treatment with FA (68). The serum of normal subjects and patients with untreated PA contains significantly more material with "folic acid" activity than patients suffering from non-Addisonian PA (69). The nature of the active material is unknown. The authors suggest, without supporting evidence, that growth may arise from folic acid polyglutamates The material is unlikely to be a simple derivative of naturally occurring FA, for injected pterylglutamic acid and CF are very rapidly cleared from the plasma of patients with severe PA (70, 71)

Excretion of formiminoglutamic acid—In the absence of adequate amounts of tetrahydrafolic acid, formiminoglutamic acid, a normal intermediary in the catabolism of histidine (72), is not metabolised to glutamic acid but accumulates and is excreted in the urine (73, 74). This occurs when the conversion of FA to tetrahydrafolic acid is interfered with by folic antagonists (75) or when there is a deficiency of FA (73). These observations have been used by Luhby, Cooperman & Teller (76) to develop a test for studying FA metabolism in patients with MA. A loading dose of 15 gm of histidine is given for 48 to 72 hr and the urinary excretion of formiminoglutamic acid is measured by a sensitive enzyme method (77) The urinary excretion of formiminoglutamic acid did not exceed 30 µg per ml in control subjects, in miscellaneous hematological conditions including hemolytic anemia and leukemia, nor in pregnancy and untreated PA In contrast, patients with non-Addisonian megaloblastic anemia who required treatment with FA excreted more than 30 µg per ml of formiminoglutamic acid in their urine (range 90 to 1900 µg. per ml) The results of this test therefore parallel the results of the *L. casei* serum assay (68) and suggest that folic acid deficiency is almost universal in MA associated with sprue syndrome, pregnancy, defective nutrition, cirrhosis, and infancy.

Tests devised for the detection of abnormalities in other metabolic pathways with which FA cofactors are concerned have not been widely used, but Butterworth and his colleagues (23) were unable to detect abnormalities of serine-glycine metabolism in patients with tropical sprue although all the patients tested excreted formiminoglutamic acid.

Plasma clearance and urinary excretion of folic acid—The metabolism of FA has also been studied by measuring its excretion in the urine after small parenteral doses (0 4 to 1 0 mg) of the vitamin (9, 70, 78) or by

from 60 to 140 μμg. per ml. [(40); *and see* (6)]. The precise significance of such levels in these patients is uncertain. Some of these patients show dramatic responses to treatment with large doses of B_{12}. This is most frequently true of patients with tropical sprue and tropical megaloblastic anemia but patients with MA of the puerperium and occasional patients with anticonvulsant MA may also respond completely or almost completely to treatment with large doses of B_{12} [(20); and see (4)] Most patients with subnormal levels in this group, however, either fail to respond or respond more slowly and less completely to treatment with B_{12}, but subsequently respond rapidly and completely to treatment with FA Nevertheless, the subnormal B_{12} levels in most of these patients probably indicate B_{12} deficiency, for many fail to absorb B_{12} or maintain a diet deficient in the vitamin (4 to 6, 48, 55 to 57). Occasionally, however, subnormal serum B_{12} levels are found in patients who apparently absorb B_{12} normally and whose diet is good (58). When these patients are treated with FA their serum B_{12} concentrations may rapidly increase to within the normal range [(40, 58), and see (59)], whereas, in uncomplicated B_{12} deficiency, the levels remain unchanged or fall when FA is given (60). It is uncertain whether the low serum B_{12} levels, which increase after treatment with FA result from tissue depletion

The serum B_{12} concentrations are usually within the normal range in MA associated with hemolytic anemia (13), cirrhosis [unless associated with a diet grossly deficient in animal protein (9)], scurvy, reticuloses and leukemia (44), and refractory normoblastic (sideroblastic) anemia (24)

Assessment of Folic Acid Deficiency

Patients with MA who have normal serum B_{12} levels fail to respond fully to treatment with small doses of B_{12}. Such patients frequently show striking hematological improvement on daily injections of 0 25 to 0 40 mg of FA or CF, or on single injections of 1 0 to 1 5 mg (9, 17, 61 to 63) On the other hand, much larger amounts of FA are required by B_{12}-deficient patients (17, 63) It has therefore been suggested that the response to smaller doses indicates FA deficiency However, patients with MA associated with hemolytic anemia and myelosclerosis require larger doses of FA (14, 17) The explanation for the increased requirements is uncertain but patients with hemolytic anemia and myelofibrosis without associated MA need more FA than do normal subjects (13, 17)

"Folic acid" levels in blood—Unfortunately, the pathogenesis of the anemia in patients who require treatment with FA is difficult to investigate because there is no method for detecting FA deficiency which is analogous to the serum B_{12} assay Various workers have measured the "folic acid" content of blood but the significance of the results is uncertain [see Chanarin (64)] Thus, Usdin, Phillips & Toennies (65) report that although whole blood hemolysates contain many substances active for *Lactobacillus casei*,

normal amounts of B_{12} (86). Absorption is normal in MA associated with pregnancy (12), cirrhosis (9, 10), and hemolytic anemia (13 to 15).

Pernicious anemia—Human gastric juice and hog IF concentrates potentiate the absorption of B_{12} in patients with PA and gastrectomy megaloblastic anemia. However, hog IF concentrates may be ineffective in patients previously treated with this material (87). It has been suggested that this refractoriness is caused by the formation of antibodies against the hog IF concentrate, for serum from these patients prevents or reduces the effect of hog IF in non-refractory patients with PA (87) as may antisera prepared in rabbits by injecting hog IF preparations (88). Occasionally, the absorption of B_{12} in a previously untreated PA is improved by human gastric juice but not by hog IF, and it has recently been reported that sera from patients with PA who have not received hog IF may nevertheless interfere with the effect of this material in PA (89). The marked hematological improvement produced by prednisolone in some patients (90 to 92) may sometimes arise from improvement in the intestinal absorption of B_{12} (91, 92). The improvement is not, however, because of increased secretion of intrinsic factor (91). It has also been claimed that large amounts of B_{12} can be absorbed by patients with PA without IF if the vitamin is given in the form of fresh, uncooked liver (93).

Malabsorption syndrome—The absorption of B_{12} is not increased by intrinsic factor in patients with intestinal malabsorption syndrome unless caused by an associated failure of IF secretion (82, 83). Normal absorption of B_{12} may be restored in idiopathic steatorrhoea by treatment with a gluten-free diet [(48); and see (82)]. Treatment with antibiotics will improve the absorption of B_{12} in certain patients with chronic tropical sprue and anatomical lesions of the small intestine [see (82, 83)]. The absorption of B_{12} may sometimes be improved in tropical sprue, and more rarely in idiopathic steatorrhoea, simply as a result of hospitalisation and prolonged treatment with B_{12} and folic acid (6, 48)

In patients who improve after antibiotics, malabsorption is presumably caused by microorganisms in the small intestine, for absorption returns to normal within a few days of starting treatment [(52); and see (82)] Indole and indoleacetic acid inhibit the utilisation of B_{12} in a mutant of *E coli* (94), and since these substances can be produced by intestinal bacteria they may be contributing factors in the MA of loop syndrome and chronic tropical sprue Antibiotics will improve the absorption of B_{12} only if the ileum is present and not short circuited, presumably because under physiological conditions B_{12} is absorbed in this area [(52); and see (82)] When very large doses of B_{12} are given, absorption may occur in the jejunum (95, 96).

The cause of B_{12} malabsorption in idiopathic steatorrhoea is uncertain but it is suggested that diversion of calcium by fatty acids may interfere

measuring the rate at which intravenous doses of CF or FA are removed from the plasma (70, 71). Patients with moderate or severe MA, with the exception of those in whom the anemia is caused by FA antagonists or by treatment with primidone (19), remove the injected FA from the plasma at a rate far exceeding the normal. This increased rate of clearance is not attributable to increased urinary excretion, for these patients also retain more of the injected dose than do normal subjects (70). The rate of removal of CF and FA was as rapid in moderate or severe PA as in non-Addisonian MA (70, 71). The conclusion that the tissues of patients with untreated PA are depleted of FA as well as of B_{12} is supported by Girdwood's observation (41) that the amount of "folic acid substances" in the liver of a patient who died with untreated PA was between only one-third and one-sixth of the amount found in control subjects, and also by the fact that the FA content of the liver of B_{12}-deficient sheep is much lower than is that of normal sheep (79).

Although the results of the clearance tests, which suggest FA deficiency, correlate well with results of *S. faecalis* assay in the megaloblastic anemias [see (66)], they do not parallel the results of either the formiminoglutamic acid excretion tests or the modified *L. casei* serum assay. A possible explanation of the difference is that in PA, in contrast to pure FA deficiency states, deficiency of FA is present only in hemopoietic tissue. The demands of this tissue may be great enough to increase the rate of clearance of injected FA and may gradually deplete the liver but because the liver is in the path of absorbed FA the depletion may rarely be severe enough to interfere with the catabolism of histidine and cause an increased excretion of formiminoglutamic acid.

An increased rate of clearance of injected folic acid is found not only in patients with MA but may occur in normal women in late pregnancy, particularly if they have twins (80), in hemolytic anemias except paroxysmal nocturnal hemoglobinuria (13), in leukemia, myelofibrosis, Hodgkin's disease, and reticulosarcoma (17) In these conditions MA may develop in the absence of dietary deficiency or intestinal malabsorption. It presumably occurs because there is an increased demand for FA by the active tissues, a demand which is not completely met by the normal daily intake of FA The fact that patients with active proliferating marrows are more susceptible to the folic acid antagonist, pyrimethamine, supports this view (81)

Absorption of Radioactive B_{12}

The use of cobalt-labelled B_{12} for absorption studies has recently been reviewed (82 to 84). Malabsorption of B_{12} is invariable in PA and in megaloblastic anemia associated with gastrectomy, anatomical lesions of the small intestine, and fish tape worm (85). Thirty to 50 per cent of patients with idiopathic steatorrhoea (adult coeliac disease) fail to absorb

Finch *et al.* (103)]. The abnormal erythrocytes are destroyed, on the average, at three times the normal rate The total erythrocytic activity of the marrow is often increased but the delivery of viable erythrocytes is no greater than in normal subjects (103). Part of the difference between total and effective erythropoiesis in these patients may be caused by the re-cycling of siderocytic iron to the bone marrow from the spleen (104) However, most of the difference is probably attributable to destruction of erythroid cells in the marrow (105). In spite of this intense hyperplasia, the total erythropoietic activity of the marrow is often less than that provoked in a normal marrow by a similar anemic stimulus (103).

Megaloblastic anemia and the synthesis of thymine—The defective hemopoiesis may result from a failure to synthesise and incorporate normal amounts of the pyrimidine, thymine, into DNA [see (106); and Reisner (107)]. The specific biochemical defect may be failure to add the 5-methyl group of thymine in a reaction which, in microorganisms and chick and rabbit marrow cells, is known to be under the influence of FA derivatives and perhaps of B_{12} [see (106, 107); Arnstein (108, 109); Dinning & Young (110)] This hypothesis is supported by the fact that thymine can replace B_{12} and FA in the treatment of MA whereas uracil is ineffective in FA deficiency and much less effective in B_{12} deficiency (106), as are also uridylic acid and orotic acid [see Rundles & Brewer (111)] The observation that the erythrocytes and leucocytes in untreated PA contain increased amounts of enzymes involved in pyrimidine synthesis (112) also suggests that there is a block in pyrimidine synthesis in MA; the accumulation of such enzymes perhaps being analogous to that found in bacteria with genetic blocks in pyrimidine synthesis (113).

In some animal and avian enzyme systems the acceptor substance for the methyl group of thymine is deoxyuridine [see (107); and Davidson (114)] which is converted into sodium thymidine As Reisner (107) points out, this constitutes a biochemical pathway whereby the uracil (as deoxyuridine) of cytoplasmic RNA could be converted into the thymine (as sodium thymidine) of DNA. He suggests that under normal conditions the extra DNA required for mitosis is built up from the constituents of RNA When there is deficiency of B_{12} or FA this transformation of deoxyuridine to thymidine proceeds slowly so that interphase is prolonged and mitosis delayed. The primitive cells therefore mature slowly and RNA accumulates in their cytoplasm

However, there is still very little direct evidence that MA in man arises from a specific defect in DNA synthesis Thus, the mean DNA content per cell in MA marrows is increased and is as great as in conditions with marked normoblastic hyperplasia (115, 116). Furthermore, in cell suspension cultures, the interphase time of megaloblasts is not prolonged (117) and megaloblasts under these conditions synthesise at least as much DNA and considerably more RNA than normoblasts from hyperplastic marrow

with absorption. Large doses of calcium lactate improve the absorption of B_{12} in some patients while the administration of sodium EDTA depresses absorption by removing calcium (97).

Absorption of Folic Acid

The process of absorption is studied by measuring the urinary excretion or the alterations in the serum level of FA or both, after oral doses of 2 to 5 mg [see Girdwood (98); Chanarin, Anderson & Mollin (99)] It is advisable to saturate patients with preliminary injections of FA before carrying out this test (100), for even normal subjects may retain considerably more FA from a single dose of 3 to 5 mg. than from a subsequent dose given 24 to 48 hr. later.

The results with either the urinary excretion or the serum level method are similar in most megaloblastic anemias Malabsorption of FA is found in most patients with idiopathic steatorrhoea and tropical sprue, in a varying proportion of patients with Crohn's disease, and in some cases of megaloblastic anemia associated with leukemia, reticuloses, and myeloproliferative disorders (98 to 102) Patients with tropical sprue may absorb normal amounts of FA 4 to 8 weeks after the start of treatment with FA or B_{12} (102). Treatment with FA usually does not alter its absorption in idiopathic steatorrhoea

Results of the two tests differ in pregnancy, in MA of pregnancy, and in patients treated with a gluten-free diet. In a proportion of pregnant women, and in an even larger proportion of patients with MA of pregnancy, the peak serum levels of folic acid after the oral dose are much lower than in normal subjects even when the size of the dose is adjusted to allow for the presence of the foetus and for the alteration in blood volume (80). In contrast, the results of the urinary excretion test are reported to be normal (41) The absorption of FA as judged by the serum level test, is normal in most patients with idiopathic steatorrhoea when marked clinical improvement follows treatment with a gluten-free diet (99) In contrast, the results of the urinary excretion test are said to be abnormal in spite of marked clinical improvement (101)

Pathogenesis of Megaloblastic Anemia

The nature of the biochemical lesion causing megaloblastic hemopoiesis is uncertain A good deal is known about the function of FA in animal enzyme systems and in microorganisms, but it is doubtful how directly these results can be applied to man. Furthermore, the precise mode of action of B_{12} in the animal organism and the relation of its functions to those of FA are mainly a matter of speculation In this next section I have tried to summarize recent work in this field

Radioactive iron studies confirm that the anemia of B_{12} and FA deficiency is the result of inhibited and defective red cell production [see

(109) and in chick marrow cells (110), and increases the uptake of ^{14}C-formate into DNA in human megaloblasts (120) but not into the liver cells of intact chicks, rats, and baby pigs (125). It has, in fact, been suggested that B_{12} deficiency may cause MA by interfering with the function of FA and its derivatives [Welch (126)] Evidence suggesting that there may be a conditioned deficiency of FA in PA has already been mentioned. However, the normal excretion of formiminoglutamic acid in B_{12} deficiency suggests that there is no general deficiency of FA Therefore, if B_{12} exerts its effect by interfering with FA metabolism it is likely to do this in the hemopoietic tissue B_{12} appears to be able to act in hemopoietic cell levels for if it is instilled into the marrow in B_{12} deficiency states it alters the marrow at the site of injection (124, 127). Nevertheless, the remarkable avidity of the liver for B_{12} emphasises the importance of this organ in the metabolism of the vitamin. Thus, when very small injections of $^{56}CoB_{12}$ (0 02 — 0 04 µg) are given to patients with severe pernicious anemia, the uptake by the liver is as rapid and complete as in normal subjects (128) In view of the close relation between B_{12} and the liver, it is interesting to learn of a recent suggestion offering the postulate that B_{12} may exert its effect through a specific action on the biosynthesis of proteins from amino acids, and that its effect on other enzyme systems may be indirect (129).

Active forms of B_{12}.—B_{12} in the liver is not in the form of cyanocobalamin for the cyanide group is replaced by adenine (130). It is not yet certain whether the function of this material differs from B_{12} in any way. B_{12} in the liver is protein-bound but differs from B_{12} in serum or mixed with gastric juice because it is available to *Euglena* without preliminary boiling (131). Whether this is a function of the adenine-B_{12} or of the binding protein is not known.

The form in which B_{12} is absorbed from the small intestine is still uncertain. Intrinsic factor binds B_{12} (132 to 135) and can remove B_{12} from other proteins (135). When B_{12} is bound in this way absorption occurs from the middle and lower small intestine in the intact rat (136), from the ileum in man (52) and in the isolated perfused small intestine (137, 138), absorption being calcium-dependent and therefore interfered with by sodium-EDTA (97, 139) The uptake of B_{12} by liver slices is also enhanced by the presence of IF and calcium (139, 140), and there is some evidence that the *in vivo* uptake of injected B_{12} by the liver is greater in subjects who secrete IF (82). It is possible that B_{12} is absorbed into the body attached to IF but extracts of intestine contain species-specific material are able to "free" B_{12} from its binding with IF (135).

THE MEGALOBLASTIC ANEMIAS

Addisonian Pernicious Anemia

Early pernicious anemia—Pernicious anemia may be recognised before anemia or other signs of B_{12} deficiency develop (44, 46, 48). At this early

(118). In addition, megaloblasts from B_{12}- or FA-deficient patients incorporate both deoxyuridine and thymidine into DNA (119), and although B_{12} and, to a lesser extent FA, stimulate the uptake of ^{14}C-formate by megaloblasts (120), these vitamins do not increase the incorporation of deoxyuridine and thymidine into DNA (119). The interpretation of culture experiments, however, is difficult and it is possible that the human megaloblasts contain enough FA or associated cofactors to supply their growth requirements during the short and relatively stress-free conditions of these cultures. Of perhaps greater importance is the fact that thymidine, in spite of its close chemical relationship with thymine, fails to "ripen" megaloblasts in culture and does not produce a hemopoietic response in megaloblastic anemias (121).

In view of the importance of FA in the transfer of one-carbon units (108), it is surprising that thymine can replace FA so completely in the treatment of MA It may be that FA deficiency is never very severe in man so that it effects only tissues and enzyme systems that are under great biochemical stress. This might account for the fact that little or no formiminoglutamic acid is excreted in FA deficiency until a loading dose of histidine is given (76), and for the observation that the glycine-serine interconversions are still possible in tropical sprue (23).

Active forms and site of action of folic acid—In the tissues FA carries out its function in the transfer of one-carbon units as 10-formyl tetrahydrafolic acid or 10-hydroxymethyl tetrahydrafolic acid [see (122)]. 10-Formyl tetrahydrafolic acid is formed from activated folic acid by the addition of a formyl group from suitable donors in an ATP-dependent reaction or by interaction with formiminoglutamic acid; 10-hydroxymethyl tetrahydrafolic acid can be formed from 10-formyl tetrahydrafolic acid (123).

The nature and function of active forms of FA have been studied mainly in mammalian enzyme systems from liver cells It is uncertain whether primitive hemopoietic cells can take up FA and convert this to more active forms for, although both FA and CF will "ripen" megaloblasts in tissue cultures (107, 117), and will also enhance the uptake of ^{14}C-formate by normal marrow cells (120), only CF is effective in transforming megaloblasts into normoblasts when these materials are instilled directly into the marrow cavity of patients with FA deficiency (124).

Vitamin B_{12} deficiency and megaloblastic hemopoiesis.—B_{12} deficiency interferes with the synthesis of DNA in the hemopoietic tissues, gastrointestinal tract and other tissues, and the synthesis of protein in the nervous system It is unlikely that B_{12} exercises both these functions through the same or a related biochemical pathway, for severe neurological change can occur in the absence of anemia In both instances it is uncertain whether B_{12} exerts its effect directly or indirectly Thus, in the case of DNA synthesis, the results of experimental studies are contradictory. B_{12} influences directly the reduction of formate to the methyl group of thymine in lactobacilli

(109) and in chick marrow cells (110), and increases the uptake of ^{14}C-formate into DNA in human megaloblasts (120) but not into the liver cells of intact chicks, rats, and baby pigs (125). It has, in fact, been suggested that B_{12} deficiency may cause MA by interfering with the function of FA and its derivatives [Welch (126)]. Evidence suggesting that there may be a conditioned deficiency of FA in PA has already been mentioned. However, the normal excretion of formiminoglutamic acid in B_{12} deficiency suggests that there is no general deficiency of FA. Therefore, if B_{12} exerts its effect by interfering with FA metabolism it is likely to do this in the hemopoietic tissue B_{12} appears to be able to act in hemopoietic cell levels for if it is instilled into the marrow in B_{12} deficiency states it alters the marrow at the site of injection (124, 127). Nevertheless, the remarkable avidity of the liver for B_{12} emphasises the importance of this organ in the metabolism of the vitamin. Thus, when very small injections of $^{56}CoB_{12}$ (0 02 — 0 04 µg.) are given to patients with severe pernicious anemia, the uptake by the liver is as rapid and complete as in normal subjects (128). In view of the close relation between B_{12} and the liver, it is interesting to learn of a recent suggestion offering the postulate that B_{12} may exert its effect through a specific action on the biosynthesis of proteins from amino acids, and that its effect on other enzyme systems may be indirect (129).

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THE MEGALOBLASTIC ANEMIAS

Addisonian Pernicious Anemia

Early pernicious anemia.—Pernicious anemia may be recognised before anemia or other signs of B_{12} deficiency develop (44, 46, 48). At this early

stage, the only hematological abnormalities may be some macrocytosis in the peripheral blood and occasional intermediate megaloblasts and giant metamyelocytes in the marrow. Achylia gastrica is present and the absorption of B_{12} is within or just above the range found in severe PA [see (44); and (82)]. The serum B_{12} level is subnormal but generally not as low as in florid PA, the concentration usually being between 70 and 140 µµg. per ml.

Patients demonstrating these features may be found in the families of patients with PA and among non-anemic patients with achlorhydria, glossitis, unexplained macrocytosis, long-standing iron deficiency anemia or non-ulcerative dyspepsia [(32, 44, 46); and see (82)]. Some of these patients go on to develop clinically obvious PA but many remain unchanged for years and may never progress beyond this early stage [(44, 46), and see (141)]. The factors which ultimately precipitate MA and other signs of B_{12} deficiency in these patients are uncertain but the severity of the absorption defect, the quality of the diet, and the presence of conditions which increase the requirement for the vitamins must all play a part

Family studies in pernicious anemia.—Callender & Denborough (46) found no significant increase in the incidence of achlorhydria in 307 relatives of PA patients when compared with 259 control subjects of similar ages The absorption of Co-labelled B_{12} was studied in the achlorhydric PA relatives and was found to be impaired, sometimes seriously, in about 50 per cent One or more parents of seven patients with PA were examined and, of these, four either had early PA or were found to have achlorhydria associated with impaired B_{12} absorption and a low normal B_{12} level. Both parents of two patients were examined and in one parent of each patient abnormalities were found; one had early PA and the other achlorhydria and defective B_{12} absorption Similar changes were found in 12 of the 54 selected relatives on whom B_{12} absorption tests were carried out

Callender & Denborough (46) confined their radioactive B_{12} absorption studies to achlorhydric relatives. However, achlorhydria is not an essential feature of PA. Furthermore, impaired absorption of B_{12} may occur in PA relatives with "free" acid in their gastric juice (84) For this reason, McIntyre and her colleagues (141) studied the absorption of B_{12} in all the available relatives of their PA patients They found impaired absorption in 40 per cent of the relatives tested and confirmed that impaired absorption occurs in relatives with or without free acid The results suggested that this low B_{12} absorption in the families of patients with PA depended on the presence of a heterozygous gene which is inherited as a single autosomal dominant The authors suggest that the impaired B_{12} absorption predisposes patients to PA The accessory factors needed to transform this into the full gastric lesion of PA are uncertain Although the mean of the results of the Schilling tests was lower than normal in the relatives at all ages, the proportion of abnormal tests increased with

age in the group of relatives but not in the control series [see also (142)] The cause of this additional age-related depression in absorption is not clearly understood but the authors point out that it may be related to the familial factors provoking achlorhydria, which they accept as being more common in families of PA patients than in controls. However, if Callender & Denborough's (46) observation is correct and achlorhydria is as common in controls as in relatives of PA, then an important factor precipitating PA in predisposed subjects may simply be the gradual development of the "normal" depression of gastric function with age.

Pernicious anemia with "free" acid.—Addisonian PA with free acid and normal gastric mucosa occurs in young infants and children whose parents are related and in whose family there is PA [see (143, 144)]. It is possible that these children represent the homozygous state of the gene predisposing to PA This appeared to be the case in the child described by Mollin, Baker & Doniach (143), for the child's father had PA and the absorption of Co-labelled B_{12} was impaired in his mother. She absorbed B_{12} normally only when an injection of carbamyl choline chloride (Carbochol) was given simultaneously with the oral dose It is noteworthy that B_{12} absorption in the child with PA was also improved by carbamyl choline chloride as was the absorption in another similar patient (144).

Tropical Sprue and Idiopathic Steatorrhoea

The clinical and hematological features of these two conditions may resemble each other closely and in both the jejunal mucosa shows characteristic histological changes (144 to 147). Nevertheless, they appear to be distinct conditions (148) for their responses to treatment are different, and severe B_{12} deficiency and severe megaloblastic anemia are probably more common in patients with tropical sprue (5, 6, 40). In idiopathic steatorrhoea, intestinal absorption improves if gluten is removed from the diet This improvement may occur rapidly or only after many months of treatment (149) Patients with tropical sprue may be cured by treatment with FA or B_{12}, or both, if caught in the early stages In the later stages these substances relieve the clinical symptoms and restore the absorption of FA to within the normal range, but they may not cure the steatorrhoea (5, 6, 150) and the impaired absorption of B_{12} may not be improved (6). Treatment with antibiotics is usually more effective (150) but this, too, appears to be most useful in the early stages, for only partial improvement may occur in more chronic patients (48, 151)

The precise histological or cytological lesion associated with malabsorption of FA in these conditions is unknown. Abnormal FA absorption tests are found in patients with both normal and abnormal jejunal mucosae, while normal absorption results may be found after treatment in patients whose jejunal biopsies are still grossly abnormal (86, 145, 147).

Tropical nutritional megaloblastic anemia.—The relationship between

tropical sprue and tropical nutritional MA is not clear. Although the diet of these latter patients is deficient in animal protein, intestinal malabsorption may often be demonstrated (6, 56). Moreover, treatment with antibiotics is sometimes effective in restoring intestinal function to normal (6) and in relieving the anemia (152). The MA in these patients frequently seems to be precipitated by other conditions. However, patients are sometimes seen with MA who require B_{12} or FA and in whom no other cause for the deficiency can be found (153, 154).

Megaloblastic Anemia of Pregnancy

Severe megaloblastic anemia of pregnancy is common in the Tropics but is much rarer in Britain and rarer still in the United States However, mild or moderate megaloblastic anemia of pregnancy is probably not uncommon in temperate countries (1). In the Tropics, this anemia may sometimes respond to treatment with large doses of B_{12} [see (4)] but in temperate climates adequate responses are rare unless such treatment is given in the puerperium. The anemia always responds to FA but suboptimal responses to large doses of B_{12} may occur even in patients whose B_{12} levels are within the normal range (155, 156). Serum B_{12} levels may occasionally be very low in these patients but similar levels are found in non-anemic pregnant women (40, 43, 44).

The response to FA, its rapid clearance from plasma following injection (80), and the increased excretion of formiminoglutamic acid in the urine (73), all suggest that the condition arises from FA deficiency The cause of the deficiency is not clearly understood. Conditions such as dietary deficiency, chronic blood loss, increased hemolysis or intestinal malabsorption may all play a part particularly in the Tropics, but in temperate climates such precipitating factors are usually absent (1, 12).

In some of these patients the deficiency appears to result from the demands for FA during pregnancy exceeding the amount available in the normal diet This view is supported by the very rapid rate of clearance of injected FA in women with twin pregnancy, a group in whom the incidence of MA is very high [see (8)].

Megaloblastic anemia associated with anticonvulsant therapy.—This has been described most frequently in patients given primidone or phenytoin sodium (20), but it may also occur if very large doses of phenobarbitone (157) or other barbiturates are given (158, 159). B_{12} concentrations in this group are usually normal or low normal but they may on occasion be definitely subnormal [see (20, 44)]. Although patients with subnormal B_{12} levels may occasionally show good responses to treatment with B_{12}, all patients respond excellently to treatment with FA. When subnormal and low B_{12} levels are encountered, treatment with FA alone produces a prompt rise of the B_{12} level to well within the normal range in most patients (58). FA treatment is therefore the treatment of choice, and if the anticonvulsant

drug is to be continued, FA should also be given to avoid a further relapse [see (20)] Although severe megaloblastic anemia is rare, minor hematological abnormalities, such as macrocytosis, are frequently seen when these drugs are given (20). It is therefore probable that some other precipitating factor is necessary to cause the severe anemia In this connection it is interesting to find that severe megaloblastic anemia caused by anticonvulsants is very rare in the United States.

Megaloblastic anemia refractory to treatment—Megaloblastic anemia refractory to treatment with B_{12} and FA may be seen in Di Guglielmo's disease (28), as a result of a specific defect in pyrimidine synthesis (27), and in refractory (sideroblastic) anemia (24). In many of the last group of patients the megaloblastic change may be altered by the administration of FA but the patient may not respond until other vitamins are given in addition (160). The patients described by Maier (26) and Roelsen & Ohlsen (25) may be examples of such patients. The cause of the MA in most of the patients in this group is little understood. In Di Guglielmo's disease the megaloblastic change does not appear to be caused by B_{12} or FA deficiency for it is not affected by treatment with the latter (28).

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PEDIATRICS: CONGENITAL ENZYME DEFECTS[1,2]

By J W. Gerrard, D M, F.R C.P.(C) and A M Marko, M D, Ph D.[3]

Departments of Pediatrics and Biochemistry, University of Saskatchewan, Saskatoon, Canada

Garrod's Croonian lectures on inborn errors of metabolism (1) are a landmark in both medical and biochemical history, but for many years the disorders he described were looked upon as medical curiosities; it is only recently that it has been realized that they form but a small proportion of the rapidly expanding field of congenital enzyme defects A study of these defects, using modern methods of investigation, has shed new light on normal and abnormal metabolism as well as on the related field of genetics; it has also presented the clinician with therapeutic problems, most of which require solution The concepts of biochemical genetics for which Tatum and Beadle (2, 3) and Lederberg won the Nobel prize began with Garrod's inborn errors and have evolved gradually to the one gene-one enzyme hypothesis "that each gene controls the production, function and specificity of a particular enzyme." It should be noted, however, that in considering enzyme defects all that is known at present is that there is a deficiency of enzyme activity; the many theoretical reasons leading to loss of enzyme activity have been discussed by Kretchmer & Etzwiler (4) and by Snyder (5). Enzyme formation belongs to the general field of protein synthesis and involves other macromolecules, deoxyribonucleic acid (the primary genetic material) and ribonucleic acid (6 to 8). It is as yet unknown how drastic must be the alteration in the structure of the enzyme molecule to result in loss of activity; it may be only a small change, such as the substitution of one amino acid for [illegible]

nucleic acids which are so intimately involved in protein biosynthesis.

This review will, nevertheless, be confined to the most recent advances related to known congenital enzyme defects; so-called "molecular diseases," for example, the abnormal hemoglobins and disorders of renal tubu-

[1] The survey of the literature pertaining to this review was concluded in August 1959

[2] The following abbreviations will be used ATP (adenosine triphosphate); DNA (deoxyribonucleic acid); UDP (uridine diphosphate); UTP (uridine triphosphate); G-6-PD (glucose-6-phosphate dehydrogenase); GSH (reduced glutathione)

[3] We are grateful to Dr G Holman, University Hospital, Saskatoon, Saskatchewan, and to Dr J B. Sidbury, Harriet Lane Home, Johns Hopkins Hospital, Baltimore, Maryland, for their help in the preparation of this review.

lar reabsorption, such as cystinuria, will not be discussed All these disorders are discussed by Hsia (10).

ENZYME DEFECTS ASSOCIATED WITH CARBOHYDRATE METABOLISM

Glycogen storage disease or glycogenosis.—The structure, formation, and degradation of glycogen in the glycogen storage diseases has been investigated by Cori and her co-workers. Her findings were summarized in a Harvey lecture in 1953 (11), and reviewed again in 1957 (12). She classifies glycogen storage diseases into four types. In type one there is a deficiency of glucose-6-phosphatase and storage of normal glycogen in liver and kidney. In type two, in which the enzyme defect is not known, excess glycogen is found in the heart, lung, liver, muscle, and often also in the tongue; death occurs in infancy. In type three there is an absence of the "debrancher" enzyme, amylo-1,6-glucosidase; glycogen which is stored in excess in liver, heart, and muscle is abnormal in structure and contains an excess of short outer branches. In type four there is a deficiency of the "brancher" enzyme, amylo-1,4-1,6-glucosidase; glycogen has long inner and outer branches and is stored in excess in liver, spleen, and lymph glands, that is, in the reticuloendothelial system; cirrhosis of the liver supervenes, presumably precipitated by the abnormal glycogen (13). Glycogen in glycogenosis has been obtained for study for the most part from the organs in which storage occurs; Cornblath *et al.* (14), however, have shown that red cells are a more convenient source of glycogen both for the investigator and the patient.

A fifth type of glycogen storage disease has recently been described by Pearson *et al* (15) in a 19-year-old male whose strength was normal at the onset of exercise but who, with exercise, became increasingly weak and developed severe cramps; he had no muscle wasting. Biochemical and histochemical analysis of thigh muscle revealed a fourfold increase in stored glycogen and no detectable phosphorylase. Glycolysis in tissue homogenate, as indicated by lactic acid production, was markedly reduced but was raised to near normal by the addition of glucose-1-phosphate. This type of glyco-
[illegible] deficiency in [illegible]

clinically simulates the disorder found in patients with a glucose-6-phosphatase deficiency. Phosphorylase is presumably not necesary for glycogen formation, this would be in keeping with the findings of Villar-Palasi & Larner (17), and of Leloir & Cardini (18) that UDP (uridine diphosphate) glucose can donate glucose to glycogen primer.

Wagner *et al.* (19) have recently studied three patients with type one glycogenosis and have found in the serum an increase in glucose-1-phosphate, glucose-6-phosphate, and other phosphorylated intermediates; these

substances cannot enter the cell or be utilized because of the deficiency of [illegible] resembling type [illegible] of eleven [illegible] y diastase, was found at necropsy in the heart and liver. Craig & Uzman (21) have also described three patients, two of whom were siblings, who resembled patients with type one glycogenosis in being short and having marked hepatomegaly; both also had splenomegaly, and one child, who died of pericarditis, had cardiomegaly. The livers and spleens of these patients contained an abnormal polysaccharide containing three fractions. Fractions I and II consisted of a mucopolysaccharide containing glucose, galactose, and glucosamine forming a complex with peptides, while fraction II was a glycolipid containing glucose, galactose, glucosamine, neuraminic acid, and fatty acids.

No specific treatment is as yet available for glycogenosis, but Yim *et al.* (22) state that prolonged treatment with glucagon is beneficial

Pentosuria—Pentosuria has been well reviewed by Knox (23) and by Touster (24). The key to the elucidation of the enzyme defect was the finding by Enklewitz & Lasker in 1933 (25) that amidopyrine given orally to a pentosuric subject was followed by an increased output of urinary 1-xylulose; they suggested at this time that this might be caused by an increased production of D-glucuronic acid which was conjugated with amidopyrine They then gave D-glucuronic acid to three patients with pentosuria and noted that this also increased the 1-xylulosuria The conversion of D-glucuronolactone to xylulose has been studied by Touster *et al.* (26); they gave a pentosuric subject labeled glucuronolactone and were able to show that its conversion entailed the loss of the carboxyl group, the aldehyde carbon becoming the fifth carbon of xylulose. Flynn (27) also found, in a patient with pentosuria, that oral glucuronic acid caused a rise above the fasting level of the plasma xylulose Bozian & Touster, quoted by Touster (24), have more recently shown that although the administration of D-glucuronolactone causes a marked rise in the plasma xylulose in the pentosuric subject, no such rise occurs in normals This suggests that in the pentosuric person there is a block in its breakdown, caused probably by a lack of 1-xylulose dehydrogenase (24).

Glucose-6-phosphate dehydrogenase deficiency—Beutler (28) has recently reviewed the sequence of events leading to the discovery that a deficiency in the red cells of glucose-6-phosphate dehydrogenase (G-6-PD) was associated with certain drug-induced hemolytic anemias. Marks *et al* (29) have shown that this enzyme deficiency is present only in red cells and not in leukocytes or liver; they have therefore suggested that the gene at fault in this disorder controls the turnover or stability of the enzyme in the red cell, thus explaining the increased susceptibility to hemolysis of the older red cells (30). The following substances, in addition to primaquine phos-

furantoin, naphthalene, *Vicia faba*, and vitamin K (menadione sodium bisulphite); infection, too, may induce hemolysis (28, 31 to 35). Of particular importance in the Mediterranean littoral is the fact that acute hemolytic anemia on exposure to the fava bean occurs only in those with low G-6-PD and reduced glutathione (GSH) stability in their red cells. A patient with the stigma of favism has recently been given primaquine phosphate and had a typical hemolytic episode, thus demonstrating susceptibility both to the fava bean and to primaquine in the same individual (36). Of importance to pediatricians is the fact that in these patients naphthalene and vitamin K may initiate hemolytic episodes in susceptible persons. Zinkham & Childs (37) record one instance in which a negress who nibbled moth balls precipitated a hemolytic anemia in both herself and her newborn baby; wrapping a baby in clothes impregnated with moth balls may also be sufficient to initiate an acute hemolytic anemia (38). The newborn, between the ages of 4 and seventy-eight hr., is particularly susceptible to these hemolytic factors because, as Zinkham & Childs (33) have shown, at this time there is a temporary fall in GSH stability in the red cells; this is not associated with a G-6-PD deficiency. In most instances, the anemia is drug-induced but this is not invariable, for Shahidi & Diamond (39) have reported brothers with congenital non-spherocytic hemolytic anemia who had a low red cell G-6-PD activity. Zinkham & Lenhard (40) have reported additional cases.

The mode of inheritance of this defect has been investigated by Childs and his co-workers (41) who studied randomly selected negroes and their pedigrees, using the glutathione stability test. They found that among males 14 per cent were positive and among females 2 per cent were positive and 5 per cent were partially positive. An analysis of their pedigrees indicated the gene to be a sex-linked dominant with variable expressivity, fathers with the defect passing it on to half their daughters and to none of their sons; heterozygous mothers, being partially positive, pass the defect on to half their sons and to half their daughters.

Their findings have been confirmed by Gross & Marks (42) in families with drug-induced hemolytic anemias, by Szeinberg & Sheba (43) and Szeinberg, Sheba & Adam (44) in non-Ashkenazic Jews in whom there was a history of favism; and by Sansone & Segni (45) in Italian families with favism. Lohr & Waller (46) have studied a family with hemolytic anemia and slight jaundice and concluded the mode of inheritance to be carried, in this instance, by a dominant autosomal gene.

Galactosemia.—This subject has been reviewed by Bain *et al.* (47), Holzel *et al.* (48, 49), and more recently by Isselbacher (50). Following the work of Schwarz and his colleagues (51) who had shown that galactose-1-phosphate accumulated in the red cells of galactosemic patients, Kalckar,

[illegible]

have verified this by analysis of cord blood Anderson *et al* (55) have also shown *the enzyme defect to be present in cord blood by utilizing the specific* enzymatic assay devised previously (56). These tests make it unnecessary to expose the susceptible infant to the toxic action of galactose.

Patients with galactosemia do not all appear to be equally intolerant of [illegible], Isselbacher [illegible] liver an en- [illegible] dine triphos- [illegible] e activity of this enzyme is only one-seventh the activity of galactose-1-phosphate uridyl transferase; it is also less active in fetal and newborn than in adult liver, thus possibly explaining the increasing tolerance for galactose which galactosemic patients manifest as they grow older.

The detection of the carrier state has intrigued workers on both sides of the Atlantic. The galactose tolerance test, although sometimes impaired in parents of affected cases, is not sufficiently definitive to detect the carrier (58) Hsia *et al* (59) have endeavored to detect these by studying the activity of galactose-1-phosphate uridyl transferase using the UDP-glucose consumption test (56); their findings indicated that in carriers of the gene enzyme activity tended to be less than in normals, but the overlap was such that the test was difficult to interpret in any individual case. Kirkham & Bynum (60) have developed a more suitable manometric method, also using hemolyzates of human red cells and carried out so that zero-order kinetics obtain, using this method they have been able to segregate carriers from normals in most instances and to demonstrate an appreciable diminution in galactose-1-phosphate uridyl transferase activity in carriers. The comparable value of these tests is discussed by Kalckar (61) Bretthauer *et al.* (62) have further modified the UDP glucose consumption test by increasing the concentration of UDP glucose and the ratio of galactose-1-phosphate to UDP glucose, and by reducing the amount of hemolyzate and the incubation period; with these modifications they have demonstrated a clear-cut differentiation between those with galactosemia, carriers of the trait, and normals.

The toxic factor in this disease is probably galactose-1-phosphate; pigeons or chicks given a diet containing 30 per cent galactose develop a neurological disorder with ataxia (63), rats on a similar diet develop cataracts (64), and *E. coli* that are deficient in galactose-1-phosphate uridyl transferase fail to grow normally on a galactose-containing medium (65). The toxic action may be direct or it may be indirect and result from the associated inhibition of other enzyme systems (66).

ENZYME DEFECTS ASSOCIATED WITH AMINO ACID METABOLISM

PHENYLKETONURIA

Phenylketonuria has been reviewed recently (4, 67 to 72). Interest in phenylketonuria has centered around three main problems: first, the nature of the enzyme defect and the details of the deranged metabolism in phenylalanine; second, the early detection and treatment of cases; and third, the etiology and the mental defect.

Phenylketonuria: the metabolism of phenylalanine in phenylketonuria—It has been definitely established by Jervis (73) and by Udenfriend & Bessman (74) that the livers of those with phenylketonuria lack phenylalanine hydroxylase in contrast to the livers of normals (75, 76). Phenylalanine hydroxylase has been fractionated by Mitoma (77) and by Kaufman (78) and the necessary cofactors studied (78 to 80). The enzyme necessary for the conversion of phenylalanine to tyrosine may be separated into two fractions, fraction I restricted to the liver and fraction II distributed throughout other tissues. The absence of fraction I was demonstrated in phenylketonuric subjects by Wallace *et al.* (76) and by Mitoma *et al* (81); phenylalanine was converted to tyrosine only when fraction I prepared from rat liver was added to liver biopsy material from a patient with phenylketonuria. The absence of this enzyme leads to the accumulation of phenylalanine in the blood Although phenylalanine blood levels may not be high at birth (82), Horner & Streamer (83) record one case in which the phenylalanine level was already high at this time and increased greatly in the first few days of life.

An inductive increase in transaminase activity has been suggested by Meister, Udenfriend & Bessman (68, 84) to explain the increased formation of phenylpyruvate from phenylalanine. Phenylpyruvate, however, *may not* appear in the urine until the child is five to seven weeks of age (82 to 84) because of the time needed for enzyme induction (72). Phenylpyruvate can yield either phenylacetate by oxidative decarboxylation or phenyllactate by reduction (68) Extending the same arguments concerning transamination, decarboxylation, and reduction, *o*-tyrosine would form *o*-hydroxyphenylacetate; tyrosine would yield *p*-hydroxyphenyl-pyruvate, -acetate, and -lactate; and tryptophan would give indolylacetate and indolyllactate

Phenylacetate is excreted as phenylacetylglutamine (68, 84 to 86). Moldave & Meister (87 to 89) have studied the enzyme activation of phenylacetate by ATP and coenzyme A prior to coupling with glutamine An adaptive increase in enzyme was attributed to the finding that biopsy specimens of liver of a patient catalyzed the formation of phenylacetylglutamine at five times the rate of normal livers (68)

The exact mechanism of the formation of *o*-tyrosine is unknown and Dalgleish (90) has suggested that it is formed by non-enzymatic hydroxylation of phenylalanine.

Phenylketonuria: the early detection and treatment of cases.—Bickel, Gerrard & Hickmans (91, 92) were the first to demonstrate that a phenylalanine-low diet was feasible, that its administration to a phenylketonuric child of two was followed by significant mental improvement, and that discontinuing the diet was followed by mental deterioration Confirmation has come from many sources (93 to 102). On such a diet biochemical abnormalities associated with the disease are eliminated, the skin and hair darken, and seizures and electroencephalographic abnormalities subside. Appreciable mental improvement does not occur in the older severely retarded children (98); the real value of diet is in the prophylactic treatment of the biochemically abnormal but mentally normal infant. Horner & Streamer (95) and Brimblecombe *et al.* (103) have summarized their experiences treating chil-

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(94) The diet is not unpalatable, but it is monotonous Status epilepticus (98) and hypoglycemia (104) in two instances, with one death, have been reported.

Detection of heterozygous carriers may be carried out by detecting elevated phenylalanine blood levels after the phenylalanine tolerance test (105). To obtain better discrimination between carriers and normals it is preferable to express the results of blood determinations as phenylalanine: tyrosine ratios (106).

Phenylketonuria · the etiology of the mental defect.—The cause of associated mental retardation remains obscure. The studies already cited suggest that early, carefully controlled limitation of phenylalanine ingestion results in normal development Mental retardation is not normally present at birth nor was it present in two children born of one phenylketonuric woman (107) although it was present in three nonphenylketonuric children born of a sec-

epinephrine and its products would be interfered with; an increased response to epinephrine has been reported to occur in phenylketonuric subjects (111), as well as low plasma epinephrine levels (112). The adrenal glands of patients with phenylketonuria differed from normals in containing tyrosine and methionine (113). The presence of abnormal proteins (114, 115) has not been supported by other workers (116, 117).

Davison & Sandler (118) have shown that phenylpyruvate, phenyllactate, phenylacetate, and phenylalanine inhibit both 5-hydroxytryptophan and dihydroxyphenylalanine decarboxylases. As a consequence, low levels of 5-

hydroxytryptamine (serotonin) have been reported in the serum of those with phenylketonuria (119) and also low levels of urinary 5-hydroxyindoleacetic acid (120 to 122).

Alkaptonuria.—This subject has been comprehensively reviewed by Knox (123) who was able to mention briefly the studies of La Du and his colleagues (124, 125) of a patient with alkaptonuria and a hiatus hernia. When the hernia was repaired surgically, a liver biopsy for biochemical study was obtained, and La Du was able to demonstrate, for the first time in this disease, an absence of homogentisate oxidase activity, an activity which was not restored by the addition of iron salts. Homogentisate oxidase is also normally found in the kidney, but it was not possible to study its activity in this patient. Homogentisic acid in normals is broken down to maleylacetoacetic acid, and it was not without interest that the liver of La Du's alkaptonuric patient had normal levels of maleylacetoacetate isomerase even though its normal substrate was absent.

Thompson (126) has outlined the main radiological features associated with ochronosis. The relationship between the radiological changes, the deposition of pigment, and the specific enzyme defect in the older patients is still not known.

Maple sirup urine disease.—Maple sirup urine disease involves the abnormal metabolism of valine, leucine, and isoleucine The name is unfortunate, first because many are not familiar with the smell of maple sirup and, second, because it associates with our national emblem a disease in which severe mental retardation is invariable (127). Thirteen probable cases have been reported (128 to 134), and while the majority have not been exhaustively investigated the connecting thread has been a familial cerebro-degenerative disease, characterized by the excretion of urine with the odour of maple sirup or burnt sugar. The first four reported cases were siblings (129) and six came from three unrelated families, suggesting an hereditary basis for the disease. The disease has its onset soon after birth and when the baby is one to two days old the usual urinary odour becomes evident The infants develop myoclonic seizures and spasticity and progress to a state of decerebrate rigidity. Hypoglycemia has been noted (128). The electroencephalogram may be abnormal (133) Death occurs between the ages of fifteen days and twenty months

With regard to the biochemical abnormality in this disease, Westall (131) first showed that plasma and urinary levels of the branched chain amino acids, leucine, isoleucine, and valine are elevated; their levels are also raised in the cerebrospinal fluid and saliva (128) Methionine is increased while the amounts of cystine, alanine, serine, and threonine are decreased (131, 132). The urinary levels, in point of fact, are not always high (128, 134) and in the early stages of the disease the urinary amino acid pattern is normal (129, 132). Mackenzie & Woolf (128) and Menkes (135) have shown, using the dinitrophenylhydrazine reaction, that the urine contains

large amounts of α-keto acids; Menkes (133) showed by chromatography that three main dinitrophenylhydrazones of the α-keto acids could be detected The dinitrophenylhydrazone of α-keto-isocaproic acid was isolated and characterized by melting points and elementary analysis. The mixture of the dinitrophenylhydrazones was reduced to the corresponding amino acids: valine, leucine, and isoleucine. One patient excreted 252 mg. of keto acids in the urine per day prior to death; he had excreted 49 mg. per day one year earlier. Normal children excrete only 2 to 4 mg. of keto acids per day

In the case described by Smith & Strang (130), phenylpyruvic and phenylacetic acids were present A typical ferric chloride test was found and the daily urinary excretion of phenylpyruvic acid was 134 4 mg. Phenylalanine, methionine, and tyrosine were also increased in the urine. The presence of α-hydroxybutyric acid and its dimerized product in the urine are believed to be responsible for the characteristic smell (130), however, α-aminobutyric acid was not detected in the urine Mackenzie & Woolf (128) confirmed the *finding of acids in the urine and they also detected an excess of indolylacetate* and indolyllactate.

Transaminase activity for branched chain amino acids has been demonstrated in the tissues from a patient obtained at necropsy (131 to 134); the

oxidation to unsaturated acids (128). Attempts to pinpoint the lesion using leucine-1-^{14}C were not successful since control tissues obtained from a child who died from trauma failed to metabolize the amino acids.

Menkes (133) has suggested that of the four cofactors, diphosphopyridine nucleotide, coenzyme A, thiamin pyrophosphate, and lipoic acid required for oxidative decarboxylation of α-keto acids, a lipoic acid deficiency ought to be considered. Except for the involvement of these cofactors in the decarboxylation of α-ketoglutarate and pyruvate there is no experimental evidence in mammals to indicate that they are involved in oxidative decarboxylations of α-keto acids generally

There is no treatment for this disease, but Mackenzie & Woolf (128) have suggested the removal of branched chain amino acids from the diet. Menkes (135), assuming a deficiency of a cofactor, has suggested the administration of lipoic acid.

Primary hyperoxaluria—This disorder has been well reviewed by Dunn (136) and by Burke (137) Additional cases have been reported since (138

to 141) and the nature of the enzyme defect has been suggested. It is a rare disease since Archer (142) has been able to find records of only ten substantiated cases, though with less strict criteria Godwin *et al.* (139) were able to collect 25.

In normal subjects urinary oxalate (dihydrate) excretion is 10 to 40 mg. per day (143); in primary hyperoxaluria it rises to 110 to 265 mg. per day. The blood levels of oxalate are unknown as there is no satisfactory micro method for its determination (142). High levels of oxalate in the cerebrospinal fluid and in pleural effusions have been demonstrated on samples collected after death (140). Necropsy specimens of liver, skeletal muscle, heart, and kidney have also been analyzed, the kidneys and heart alone containing an excess of calcium and oxalate.

With regard to the etiology of the disorder, this has been shown by Archer (138, 142, 143) not to be attributable to increased intake or absorption of oxalate, nor is it caused by a low renal threshold for oxalate In looking for a source of oxalate, Archer (142) surmised that it might be derived from glycine, for oxalate is an end product of its metabolism arising from oxidation of glyoxylate; glyoxylate may also be derived from sugars as a two carbon fragment, from oxidation of sarcosine, and from the transamination of glycine with α-keto acids (144). Archer and his colleagues (142) found that the administration of glycine to two patients increased the oxaluria in one; conversely, the provision of a low protein (glycine) diet and later the administration of sodium benzoate which combines with glycine to form hippurate, reduced the oxaluria in both. Later, using isotopic tracer glycine-1-^{13}C, Scowen, Crawhall & Watts (145) showed conclusively that glycine was the precursor of at least some of the oxalate

An interesting attempt has been made to correlate glyoxylate metabolism and deposition of calcium oxalate crystals in different tissues (140) Metabolic activities of glyoxylate are higher in myocardium and smooth muscle generally, which may explain the presence of crystals in the heart and different arteries of the body.

Paper chromatography of urines from primary hyperoxaluria (138) have a normal amino acid pattern, although one might have expected increased amounts of glycine, since glyoxylate is a highly reactive compound and will transaminate with other amino acids non-enzymatically (144). For this reason it is unlikely that primary hyperoxaluria is related to the case of glycinuria recorded by De Vries (146); the latter was an hereditary disorder associated with nephrolithiasis and the stone consisted mainly of calcium oxalate with a small amount of free glycine Glycinuria was caused by a lowered renal threshold, the urinary oxalate excretion of which was normal.

Argininosuccinic aciduria—In 1958, Allan and his colleagues (147) described two siblings who were severely retarded mentally, both of whom excreted large amounts of a then unknown amino acid in the urine. These

two children had smiled, sat up and walked at normal ages, but had not learned to talk; the first child to be studied developed epilepsy when she was nearly three years old. Both she and her affected brother who had had no seizures had abnormal electroencephalograms, brown brittle hair and heart murmurs, possibly caused by ventricular septal defects. The unknown amino acid was later identified by Westall (148) and found to be argininosuccinic acid, an intermediate metabolite in the urea cycle. The enzyme which splits argininosuccinic acid into arginine and fumaric acid is, therefore, presumably absent.[4]

Hartnup disease—Hartnup disease, previously referred to as "Hart's syndrome" and also "H" disease (149), is characterized by hereditary pellegra-like skin rash with temporary cerebellar ataxia and a characteristic amino-aciduria Dent first described this disease in 1952 (150) after collaboration with Dr. Hart who made the original discovery. A detailed discussion of this illness is presented by Baron *et al.* (149) and previous scanty references are cited therein They described the new disease affecting four of eight children of a first cousin marriage. The clinical syndrome is complex and inconstant although the most constant feature is a tendency to develop a rash on exposure to sunlight. Reversible cerebellar ataxia may develop.

The gross amino-aciduria is of a unique pattern and is evidently of renal origin. Alanine, serine, asparagine, glutamine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, and histidine are found in excess in the urine, cystine, lysine, and glycine are moderately increased; taurine is normal while proline, hydroxyproline, methionine, and arginine are not detected Evered (151) has measured the plasma and urine concentrations of the amino acids and has calculated approximate plasma clearance of the amino acids in Hartnup syndrome as well as in other diseases.

An excess of indole derivatives is present in the urine, such as indoxyl

acetylglucosiduronic acid, but in Hartnup disease only moderate amounts of this metabolite are found in the urine. It should be pointed out that by ammonolysis the glucuronide is converted to an indolylacetamide, and such conditions are present if paper chromatography is conducted with ammoniacal solvents.

Milne *et al* (154) indicate that increased excretion of indolylacetate and indolylacetylglutamine is not as constant as was previously thought; appar-

[4] Dent has reported recently [Dent, C E., *Proc Roy Soc Med*, 52, 885 (1959)], that two affected siblings had normal plasma ureas and urine urea outputs and, therefore, a metabolic block in the formation of urea is unlikely He has suggested that the block is concerned with some hitherto unknown synthetic process in brain metabolism.

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patient without hypertension, and Cope (163) has found evidence of 21-hydroxylation in a normotensive patient. That hydroxylation can occur in the C_{21} position in adrenal hyperplasia is further suggested by the finding reported by Luetscher & Axelrad (164) of normal levels of sodium-retaining activity in the urine of patients with the salt-losing syndrome, and of Prader *et al.* (165) that patients with adrenal hyperplasia excrete aldosterone in the urine.

There are, however, different pathways for the biogenesis of corticosteroids in the glomerulosa and fasciculata and reticularis (166), and hydroxylation might well occur in the C_{21} position in the glomerulosa without *ipso facto* occurring in the same position in the fasciculata and reticularis The nature of the enzyme defect or defects is, as Birke *et al* (167) have indicated, far from settled.

Congenital goitrous cretinism —The formation and nature of the thyroid hormone or hormones have been reviewed recently (168 to 170) as has the syndrome of congenital goitrous cretinism (171 to 174). Goitrous cretins are believed to fall into three main groups: (*a*) in which there is an inability to bind iodine to tyrosine; (*b*) in which there is a lack of the coupling enzyme so that mono- and diiodotyrosines are formed but not coupled to form thyroxine; and (*c*) in which there is an absence of the deiodinating enzyme, "dehalogenase," with a consequent loss of iodide in the urine. Most cases, according to Carr *et al* (175) who analyzed thirty consecutive cretins of whom five were goitrous, fall into group (*a*). Gardner *et al.* (176) have made a careful study of six cases: two could trap but not organify iodide and fall into group (*a*); two superficially seemed to lack a dehalogenase but it was considered that there was an associated defect in the synthesis or release of thyroxine; two could organify some iodide, and in them the defect appeared to be partial The latter suggestion is in keeping with the observations of Clayton *et al* (177) of another family

Not all patients with goitrous cretinism fall into the above categories. DiGeorge & Paschkis (178) described one patient in whom a biologically inactive iodinated protein was present in the serum Stanbury & McGirr (171) have also seen two similar patients DeGroot *et al* (179) report a fourth who could trap iodide form mono

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(180) may fall into the same category. DeGroot & Stanbury (181) have summarized their experience with this group of patients, they suggest that the thyroids of these patients make or secrete an inactive polypeptide related to thyroglobulin.

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ently indolylacetate excretion is influenced by urinary pH. From the results of feeding tryptophan to patients with Hartnup disease the authors suggest there is a defective transport of tryptophan in the cells of the proximal renal tubules, jejunum, and possibly the liver. An abnormally small amount of injected tryptophan is oxidized to formylkynurenine by tryptophan peroxidase in the disease, but there is no proof that there is an actual deficiency of this enzyme and the defect may arise from reduced contact of enzyme and substrate Baron and his colleagues (149) suggested that there might be a metabolic blo[illegible]
or an abnormality [illegible]

Albinism.—On[illegible]
fects is that associated with albinism which has recently been reviewed by Knox (155). The lack of pigmentation in this disorder is probably caused by an absence of tyrosinase in the melanocytes, but this has not yet been proven.

ENZYME DEFECTS IN HORMONAL SYNTHESIS

Congenital adrenal hyperplasia.—Bartter *et al.* (156) first suggested that because ACTH stimulation caused an increased output of 17-ketosteroids only, congenital adrenal hyperplasia resulted primarily from an inability of the adrenal gland to synthesize "glucocorticoids." They suggested that there was a secondary increase in ACTH production, later shown to be true by Sydnor *et al.* (157), and that this was responsible for the high output of androgens. Jailer (158) then suggested that the disorder was an inborn [illegible]

and suggested that the prime defect in the disease was in the conversion of 17-hydroxyprogesterone to hydrocortisone, that is, in the 21-hydroxylase. There are, however, three forms of the disease. In its simplest form there is only virilization, in 33 per cent there is also a salt-losing tendency, and in 6 per cent there is hypertension (160), it would not be expected that the same enzyme defect or defects would occur in all three froms

In 1956, Eberlein & Bongiovanni (161) studied a patient with an associated hypertension Compound S and tetrahydro S, but no 11-oxygenated C_{21} or C_{19} steroids were found in the peripheral blood; tetrahydro S was also found in the urine. It was assumed in this instance that there was an absence of the 11β-hydroxylase, the 21-hydroxylase being present. The hypertension was thought to result from the presence of deoxycorticosterone in the blood for its reduced metabolite pregnane-3α21-diol-20-one was present in the urine The elegant suggestion that a deficiency of 21-hydroxylase leads to the common form of adrenal hyperplasia, and a deficiency of an 11β-hydroxylase to the hypertensive form, lacks confirmation for Gandy & Keutmann (162) have found evidence of an 11β-hydroxylase deficiency in a

Hepatolenticular degeneration (Wilson's disease).—Wilson's disease has recently been reviewed by Bearn (197), Bickel (198), and Walshe (199). The hallmark of this disease is a low plasma ceruloplasmin (200). Scheinberg & Gitlin (201) originally suggested that the disease could be compared to hemophilia in that both are specific protein deficiencies, in which case it should be considered a "molecular disease." Holmberg & Laurell (202) had earlier shown that *in vitro* ceruloplasmin can act enzymatically as a copper oxidase, and Scheinberg & Morell (203) have suggested that it acts enzymatically *in vivo* by releasing copper in the villous capillaries of the intestine, thus inhibiting copper absorption. If this is one of its main functions Wilson's disease should be considered to be attributable to a genetically determined enzyme defect. The administration of ceruloplasmin has not, however, according to Bickel *et al.* (204), led to any amelioration of the disease but only severely affected cases were treated Curzon (205) has discussed in detail the structure and function of ceruloplasmin.

The most important recent advance in the treatment of this disease has been the introduction of penicillamine by Walshe (206, 207) Treatment is simplified for the drug is given orally; it causes a marked cupruria. Its use has been commented on favourably by Fister *et al.* (208) and by Seven *et al* (209), but there is a danger of a granulocytopenia developing, probably on account of its antagonism to pyridoxine which should therefore be given in addition (199). As treatment may halt the progress of the disease it is important to detect asymptomatic siblings of known cases as Lygren *et al.* (210) and Bickel (198) have done. [illegible]

[illegible] cases, conversely Vella (213) has reported two subjects who were clinically normal but who had low ceruloplasmin plasma levels Individuals heterozygous for the trait are reported to have normal ceruloplasmin levels by Bearn (211) and Scheinberg *et al* (214), but Grouchy (215) states that it is possible to identify carriers by their intermediate ceruloplasmin levels. Serum copper levels are also normal in carriers of the gene (216).

Wilson's disease, particularly in the young, may present with evidence of hepatic cirrhosis only (217), there may be an associated portal hypertension caused probably, according to Taylor *et al* (218), by obstruction to blood flow at the presinusoidal level. The diagnosis in these cases may be difficult (219) for cupruria may be associated with hepatic cirrhosis only (220). Postmortem analysis of the copper content of livers from children dying of juvenile cirrhosis may show it to be as great as in cases of Wilson's disease; the silver content in both instances is also high but the copper content of the brain in these cases has been shown by Butt *et al* (221) to be normal. The deposition of copper in the brain in Wilson's disease has

(182, 183). It is not surprising, therefore, that the location of the enzyme defect has not been identified in all cases of goitrous cretinism. A report by Zondek *et al.* (184) that administration of 90 mg. of triiodothyroacetic acid over a nine-day period not only brought the protein-bound iodide to normal in one goitrous cretin, but made her euthyroid for six months, should be treated

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ally and the case reported by Hutchinson, Arneil & McGirr (185) which did not respond to thyroid but which improved dramatically when given 100 mg. of tri-iodothyronine may be an example of such a case.

OTHER ENZYME DEFECTS

Hypophosphatasia

The syndrome associated with a low alkaline phosphatase was first reported by Rathbun (186). Currarino *et al.* (187), Fraser (188), Swoboda (189), and Dickson & Horrocks (190) have recently reviewed the published cases adding others of their own. Cases fall into three main groups: (*a*) those who already have severe manifestations of the disease at birth, death usually occurring in infancy; (*b*) children in whom the lesions gradually appear after the age of six months, many of whom survive; and (*c*) adults in whom the disorder is detected incidentally as when a survey is made of relatives of a known case. The enzyme defect is widespread and phosphatase activity has been shown at necropsy to be diminished in liver, kidney, small intestine, clavicle, femur, and skull (191). Kretchmer *et al* (192) have more recently demonstrated an absence of alkaline phosphatase in the leukocytes of a patient with this disease The urine of affected cases contains phosphoethanolamine (193, 194) as does that of some relatives, but the significance of this finding is not certain for phosphoethanolamine has also been found in the urine of children with celiac disease (195), scurvy, and hypothyroidism (188). It is possible that phosphoethanolamine is a substrate for alkaline phosphatase (193, 194) The only other abnormal substance to be reported in the urine is adenosine monophosphate (191)

Hypophosphatasia: treatment.—Vitamin D in large doses has been tried (196) but its use is probably contraindicated because many patients already have hypercalcemia and this may be aggravated. Cortisone has been found to be beneficial in one case (188). The administration of cortisone in low dosage to this patient was followed by radiological evidence of healing and a rise in the serum alkaline phosphatase; the withdrawal of cortisone was followed by a fall in the alkaline phosphatase and the development of fresh radiological lesions in the epiphyseal plates where growth was occurring. It would seem that a trial of cortisone therapy is indicated for it need not be continued if it does not prove beneficial. Swoboda (189) has given magnesium gluconate, 1 to 2 gm. daily, without benefit.

glucuronidation of bilirubin though Schmid & Hammaker (236) have concluded that glucuronidation is not impaired, for conjugation of N-acetyl-*p*-aminophenol is carried out normally; they suggest that in this disease there is defective transport of bilirubin from the plasma to the liver cell In the [illegible]

[illegible] and it is for this reason that hyperbilirubinemia is so frequent in the newborn (238). Studies by Brown & Zuelzer (239) in the fetal, newborn, and adult guinea pig, have demonstrated not only markedly reduced glucuronyl transferase activity in the newborn, but also defective formation of glucuronic acid from glucose.

Another defect of great interest, but of great rarity, is acatalasemia described by Takahara (240). One of the most recently described disorders is "cystathioninuria." This has been discussed briefly by Harris (241). It is probable that many diseases and disorders will eventually be classified as enzyme defects. The future of biochemical genetics as applied to medical problems has been indicated by Hsia (10).

been studied by Porter & Folch (222) who found that the copper is bound to protein in a form not present in normal brain, and reacts directly with diethylthiocarbamate (223).

Bearn and his colleagues (224) have studied renal function in cases of Wilson's disease and concluded that disturbances of function were probably secondary to the accumulation of copper in this organ. Finby & Bearn (225) consider that the roentgenologic changes sometimes found in these patients, namely osteomalacia, bone fragmentation, fractures, and arthritis, though similar to those found in the Fanconi syndrome, are not related to the severity of the renal disease but are probably linked with the deranged copper metabolism. Bearn & McKusick (226) have reported the presence of azure lanulae in two patients with Wilson's disease. Ezzo *et al.* (227) have reported another asymptomatic case with acanthosis nigricans

Pseudocholinesterase deficiency.—This enzyme deficiency was brought to light by the use of succinylcholine as a muscle relaxant in anesthesia Kalow & Staron (228) have studied, using dibucaine, a local anesthetic inhibitor of pseudocholinesterase, the incidence of pseudocholinesterase deficiency in a population of 1556. They found two with very little pseudocholinesterase, 59 with intermediate, and 1495 with normal activity. Five hundred and forty of their subjects were healthy, an analysis of these suggested the frequency of the gene to be 0 0140–0.0036, and the incidence of homozygotes to be approximately 1 in 5100. They studied in detail two families carrying the defective gene and concluded it to be recessive and autosomal.

Glucuronyl transferase deficiency—Crigler & Najjar (229) in 1952 described an inbred group of families in which there were seven children with a congenital non-hemolytic jaundice In 1956, Childs & Najjar (230) gave further details of two survivors and in the same year Rosenthal *et al.* (231) described an additional unrelated case. Glucuronyl transferase activity in this disease has been studied by Schmid *et al.* (232) in three patients who were virtually unable to conjugate bilirubin with glucuronic acid; conjugation of salicylates, hydrocortisone, and menthol was also markedly impaired Childs *et al* (233) have also studied glucuronyl transferase activity in their two patients as well as in several near relatives, and have likewise concluded that they were unable to conjugate bilirubin with glucuronide because of a defect of glucuronyl transferase activity; they found that one subject studied had a partial deficiency in the conjugation of reduced hydrocortisone with glucuronic acid, while another showed a like defect in the conjugation of trichlorethanol More extensive studies with sodium salicylate showed a marked defect in conjugating this substance; several near relatives were unable to conjugate it as efficiently as normals. These findings suggest that the enzyme glucuronyl transferase lacks specificity in the human, in Gunn rats, according to Arias & Johnson (234), it does not

In Gilbert's disease, Arias & London (235) have also found defective

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SPECIAL THERAPEUTICS: PHYSIOLOGY OF DIURETICS[1]

By Karl H. Beyer, Jr., M.D., Ph.D.
Merck Institute for Therapeutic Research, West Point, Pa.

The management of fluid retention associated with cardiovascular-renal diseases of primary or secondary origin probably has advanced more enthusiastically in the past 40 months than it has in the 40 years since the clinical observation of the diuretic action of the organomercurials made by Saxl & Heilig (1). A number of excellent review-type articles pertaining to the basis for the renal regulation of electrolyte balance by diuretics or saluretic agents have appeared in the past few months. Although the list is by no means complete, one may profitably refer to the recent articles by Mudge (2) on patterns of tubular dysfunction, Berliner *et al.* (3) on the action of antidiuretic hormone, and Pitts (4) on the action of diuretics in the May 1958 issue of the *American Journal of Medicine*. The December 1958 issue of *Archives of Internal Medicine* carries a symposium on recent advances in the knowledge of the causes of edema and in diuretic therapy, including articles on the regulation of water balance by Berliner (5), on aldosterone by Lieberman (6) and aldosterone antagonists by Liddle (7); on non-mercurial diuretics by Beyer (8) and mercurial diuretics by Ray (9). The February 1959 issue of the *American Journal of Cardiology* is devoted, for the most part, to studies on diuretic therapy and antihypertensive therapy, and among the several excellent articles is included the treatment of hypertension of ambulatory patients by Moyer & Beem (10). Whereas the February 3, 1958 number of *Annals of the New York Academy of Sciences* is devoted to a number of papers dealing with basic principles and the clinical application of diuretic agents, as edited by Taggart (11), the August 1959 issue of *International Record of Medicine* contains the most recent symposium on chlorothiazide and its derivatives, as edited by Moyer (12). Since a systematic discussion of mercurial diuretic agents is not included herein, for lack of space, the reader is referred to the current reviews of this subject by Ray (9) and by Mudge & Weiner (13).

The common denominator for the aberrant interaction of the multiple extrarenal and renal factors that may contribute to the retention of electrolytes and water is the imbalance of glomerulotubular function (Fig. 1). The pathogenesis of chronic congestive heart failure can be envisaged to result from such an accumulation of fluid as a glomerulotubular imbalance could engender. Thus, if glomerular filtration is reduced to a greater degree by disease than is the tubular capacity to reabsorb salt and water, the percentage reabsorbed increases and the amount excreted diminishes.

[1] The survey of the literature pertaining to this review was concluded in August, 1959

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The common denominator for the aberrant interaction of the multiple extrarenal and renal factors that may contribute to the retention of electrolytes and water is the imbalance of glomerulotubular function (Fig. 1). The pathogenesis of chronic congestive heart failure can be envisaged to result from such an accumulation of fluid as a glomerulotubular imbalance could engender. Thus, if glomerular filtration is reduced to a greater degree by disease than is the tubular capacity to reabsorb salt and water, the percentage reabsorbed increases and the amount excreted diminishes.

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This contributes to the expansion of blood volume, the accumulation of fluid in the extracellular space, and to ascites (14, 15).

The critical element in this imbalance, as it pertains to the pathogenesis of edema of chronic heart failure, is the retention of sodium. The reason for this is that the ion exchange of sodium in the glomerular filtrate for cellular hydrogen (and potassium) ions across the luminal cell membrane of the tubule is an active process, at least insofar as hydrogen ions are made available for exchange; as by the action of carbonic anhydrase (16, 17). Since chloride is the predominant anion in the filtrate, it is (passively) reabsorbed or diffuses to the greatest extent with sodium. The increased salt reabsorbed retains with it an osmotic equivalent of water. Thus, the retention of water is of a secondary nature, being determined by the increased reabsorption of salt. As sodium accumulates in the body it tends to

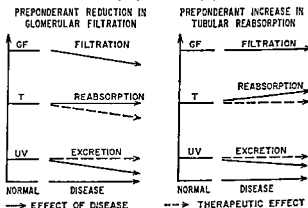


FIG. 1 Effect of renotropic agents on the excretion (UV) of electrolytes and water that has been diminished by glomerulo (GF)-tubular(T) imbalance

exchange with potassium in the cells (18) Thus, under these circumstances the retention of sodium (i e, the expanded sodium volume) may exceed that of water, even though the plasma sodium level may be normal (19 to 22).

The glomerulotubular imbalance may be occasioned by an increased reabsorption of salt without a notable change in glomerular filtration rate This may be induced by the elaboration of abnormal amounts of adrenal corticoids (23, 24), particularly aldosterone (25 to 27), or iatrogenically, especially by the administration of the earlier antiinflammatory steroids More subtly, neurogenic or adrenergic stimulation or increased venous pressure may cause an increased reabsorption of salt and water without necessarily affecting glomerular filtration rate (28 to 30).

The therapeutic role of a diuretic agent is to induce a more harmonious

relationship between glomerular filtration and tubular reabsorption of electrolytes and water (see Figure 1). This it does by diminishing the tubular reabsorption of water in one manner or another, for neither a single principle nor mode of action applies to the several categories of compounds that are generally classified as diuretic agents Thus, the term "diuretic agent" applies to all compounds that cause a net increase in the volume of urine elaborated per unit time, regardless of composition. The term "saluretic agent" connotes one which primarily increased the excretion of sodium chloride Under conditions of fluid retention the saluretic agent may induce diuresis.

Several classes of diuretic agents that are available or are likely to be made available to the physician are included in the following sections. Since each category will be considered from a basic point of view, the attributes of all the compounds will not be recited in detail.

OSMOTIC DIURETIC AGENTS

Osmotic diuretic agents are compounds that are filtered by the glomeruli, are poorly reabsorbed by the renal tubules and, consequently, inhibit the reabsorption of an osmolar equivalent of water. The hexahydric alcohol, mannitol, is a classical example of such a compound (31) (Fig. 2) This agent is ultrafiltered completely at the glomeruli and is not reabsorbed. These attributes have made it a useful agent for the measurement of glomerular filtration rate (32).

Actually, all diuretic agents except water (33) and perhaps ethyl alcohol (34), act basically like mannitol Whereas mannitol provides its own filtered solute in sufficient amount to withhold water osmotically from reabsorption by the tubules as it passes down their lumina, most other agents act in whole or in part by inhibiting electrolyte reabsorption in

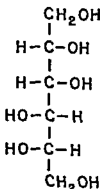
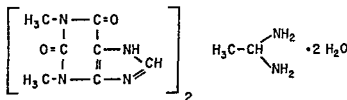


Fig. 2 Structural formula of mannitol

order to acomplish an impedance of the back diffusion of water. The characteristics of mannitol diuresis and that induced by the other classes of compounds to be mentioned become divergent beyond that point Mannitol causes an increased water output without a substantial reduction in the reabsorption of sodium, potassium or chloride ions. Likewise, it has no effect on glomerular filtration or urinary pH. (35, 36). Thus, it has no important action in reducing the excessive accumulation of sodium which usually is responsible for the retention of fluid. This fact, associated with the inconvenience of intravenous administration of large amounts of material having necessarily a short duration of action, has minimized its importance or general usefulness.

Many other compounds have been employed as osmotic diuretic agents Among these, urea and ammonium chloride come to mind. Whereas urea



AMINOPHYLLINE

(THEOPHYLLINE ETHYLENEDIAMINE)

FIG 3. Structural formula of theophylline ethylenediamine

can be administered orally to some patients, it is reabsorbed partially by the renal tubules and is not generally useful when employed in dosages that can be tolerated

Ammonium chloride functions at least partially as an osmotic diuretic agent in that it supplies excess chloride ion to the glomerular filtrate While a portion of the ammonium ion is excreted, ammonium chloride usually increases the acidity of the urine. It is more frequently employed as interim therapy in the intermittent administration of organomercurials, since they are more effective under conditions of moderate (ammonium chloride-induced) acidosis.

XANTHINE DIURETICS

Until the past few years, the xanthine diuretics were among the most generally used and least understood drugs of this broad category. Theophylline ethylenediamine (Fig. 3) has been most frequently employed, but none of these compounds is of a sufficient order of potency to be very useful where a diuretic agent is indicated

When administered, especially intravenously, these compounds can

cause an increased glomerular filtration rate (37, 38), presumably because of their cardiotropic effect on renal hemodynamics. In the early literature on diuresis this was held to be their mode of action, until Cushny & Lambie (39) and later Walker *et al* (40) showed that the diuretic effect of these compounds obtained even though glomerular filtration rate was not elevated Thus, their effect on glomerular filtration no longer is held to be an important component of their diuretic action (41 to 43).

Actually, the primary diuretic action of the xanthines is to inhibit the renal tubular reabsorption of sodium and chloride (33, 43 to 45). This effect is similar qualitatively to that of chlorothiazide and its analogues and to that of the mercurials, in that substantially effective dosages may also cause some increase in potassium excretion. However, these compounds have a relatively very low order of potency.

The mode of diuretic action of the xanthines is not at all understood. The basic effect is probably quite different from that of any of the several classes of compounds to be discussed, except possibly the aminouracils These two classes of compounds share the pyrimidine moiety in their chemical structures (compare Figures 3 and 4).

PYRIMIDINEDIONES OR AMINOURACIL DERIVATIVES

A discussion of the pyrimidinediones or aminouracils logically follows that of the xanthines, to which they are comparable in action and potency These compounds resulted from a structure-diuretic activity study which was first reported for the isocytosines by Van Arman (46) and which evolved to a greater emphasis on the aminouracils which had been described by Papesch & Schroeder (47). Two compounds of clinical importance evolved from this program· aminometradine[2] and amisometradine[3]

Kattus *et al* (48) were first to publish their preliminary studies on aminometradine. In clearance experiments on dogs, they found that the compound diminished the tubular reabsorption of sodium and chloride ions without necessarily influencing renal hemodynamics. There was induced a lesser increase in potassium excretion From their experiments, they concluded that the increased urine flow was secondary to the inhibition of sodium (chloride) reabsorption They extended their studies to demonstrate the same qualitative findings in normal man and in edematous patients. Their findings were corroborated by Caccamo *et al.* (49), Clark & Hagans (50), Platts & Hanley (51), and others The order of activity for aminometradine was sufficient to be useful, but the incidence of nausea and gastrointestinal distress was substantial in some series and as reported by Nissen & Zachau-Christiansen (52).

[2] The trade name of G D Searle & Co. for aminometradine, 1-allyl-3-ethyl-6-aminotetrahydropyrimidinedione, is Mictine.

[3] The trade name of G D. Searle & Co for amisometradine, 1-methallyl-3-methyl-6-aminotetrahydropyrimidinedione, is Rolicton.

order to acomplish an impedance of the back diffusion of water. The characteristics of mannitol diuresis and that induced by the other classes of compounds to be mentioned become divergent beyond that point. Mannitol causes an increased water output without a substantial reduction in the reabsorption of sodium, potassium or chloride ions. Likewise, it has no effect on glomerular *filtration or urinary pH (35, 36). Thus, it has no* important action in reducing the excessive accumulation of sodium which usually is responsible for the retention of fluid. This fact, associated with the inconvenience of intravenous administration of large amounts of material having necessarily a short duration of action, has minimized its importance or general usefulness.

Many other compounds have been employed as osmotic diuretic agents. Among these, urea and ammonium chloride come to mind. Whereas urea

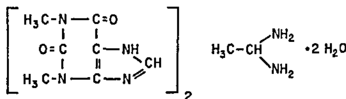


FIG. 3. Structural formula of theophylline ethylenediamine.

can be administered orally to some patients, it is reabsorbed partially by the renal tubules and is not generally useful when employed in dosages that can be tolerated

Ammonium chloride functions at least partially as an osmotic diuretic agent in that it supplies excess chloride ion to the glomerular filtrate While a portion of the ammonium ion is excreted, ammonium chloride usually increases the acidity of the urine It is more frequently employed as interim therapy in the intermittent administration of organomercurials, since they are more effective under conditions of moderate (ammonium chloride-induced) acidosis.

XANTHINE DIURETICS

Until the past few years, the xanthine diuretics were among the most generally used and least understood drugs of this broad category Theophylline ethylenediamine (Fig 3) has been most frequently employed, but none of these compounds is of a sufficient order of potency to be very useful where a diuretic agent is indicated.

When administered, especially intravenously, these compounds can

(chlorazinil[4] and amanozine) that currently have received careful laboratory and clinical appraisal. Their structures and that of formoguanamine are presented for comparison in Figure 5.

Certain of the triazines are among the more potent of diuretic agents in the rat, as judged by the minimal effective dose. However, Kagawa & Van Arman (69) have pointed out that they are apt to be effective over a relatively narrow dose range, at least in laboratory animals. Various examples of the triazines differ in their comparative effects on salt and water excretion, but it would appear that they generally cause a relative increase in water excretion over that of salt.

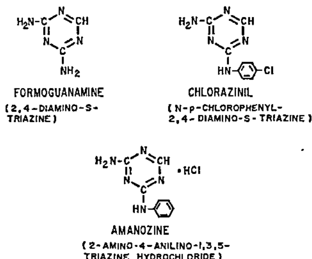


FIG 5 Structural formulae of representative triazines

Chlorazinil, originally described by Clauder & Bulcsu (70), has been subjected to considerable study because of its potency and utility. As a representative triazine, it illustrates the positive and negative attributes of this class of compounds. Williamson *et al.* (71) and LeSher & Shideman (72) have described the varied effects of the compound in rats. As the dosage of the compound administered to rats that had been given an oral load of isotonic saline is increased, there is a progressive increase in salt and water excretion. Excretion of potassium, which is depressed at low dosage, returns to control values as dosage is increased. However, if hydration is accomplished with water instead of saline an increased excretion of salt can be induced, but they report an accompanying antidiuretic response with no change in potassium excretion.

[4] The trade name of Riker Laboratories for chlorazinil, N-*p*-chlorophenyl-2,4-diamino-s-triazine, is Daquin.

A closely related compound, amisometradine (Fig 4), has been shown to be essentially as effective and less offensive to the gastrointestinal tract A substantial literature attests to the utility of both aminometradine and amisometradine in the management of the edema of congestive heart failure (53 to 55), of cirrhosis (56), and of toxemia of pregnancy (57)

Ford *et al* (58) have compared both these compounds and theophylline ethylenediamine, administered orally, with intramuscularly injected meralluride. They found amisometradine and theophylline ethylenediamine to be 0 5 times and aminometradine to be 0 7 times as active as the organomercurial Most investigators have considered the order of potency to be sufficient to maintain an edema-free state in perhaps half of the ambulatory cardiac patients without serious disturbances of electrolyte balance In

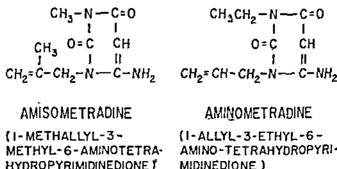


FIG 4 Structural formulae of representative pyrimidinediones

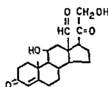
other patients its effect was supplemented with mercurials (59 to 61). The gastrointestinal complaints and nausea remain the most frequent side effects Karrel (62) attributed a case of thrombocytopenia purpura to aminometradine. On the whole, these compounds represent an advance in oral diuretic therapy over the xanthines

TRIAZINES

In 1944, Lipschitz & Hadidian (63) reported that among a series of some 70 amides and amines, melamine, adenine, and formoguanamine were the more potent diuretic agents for the rat Lipschitz & Stokey (64) confirmed the increasing order of activity of these three compounds in the dog when administered orally. They noted an increased excretion of both salt and water, but presumably the osmolarity of the urine was reduced The diuretic action of formoguanamine has been confirmed in the dog (65) and in man (66, 67) The first of these reports by Ludwig (68) indicated that formoguanamine was a reasonably safe and effective oral diuretic for man when administered in a dose of one gram.

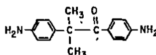
Out of a considerable amount of chemical and biological effort in a number of laboratories have come at least two compounds of this class

Luetscher *et al*, served to identify this as the most potent of adrenal cortical hormones with respect to the renal regulation of sodium and potassium excretion. The demonstration of its utility for the maintenance of the Addisonian patient by Mach *et al.* (83) and the description of the clinical syndrome of hyperaldosteronism by Conn (84), further emphasized the fundamental importance of aldosterone. Whereas aldosterone causes a decreased excretion of sodium (chloride) and an increased elimination of



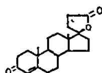
ALDOSTERONE

(11β, 21-DIHYDROXY-3, 20-DIKETO-4-PREGNENE-18-AL)



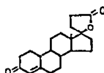
AMPHENONE B

[1,2-BIS-(p-AMINOPHENYL)-2-METHYL-1-PROPANONE]



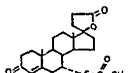
SC-5233

[3-(3-OXO-17β-HYDROXY-4-ANDROSTEN-17α-YL)-PROPIONIC ACID γ-LACTONE]



SC-8109

[3-(3-OXO-17β-HYDROXY-19-NOR-4-ANDROSTEN-17α-YL)-PROPIONIC ACID γ-LACTONE]



SC-9420

[3-(3-OXO-7α-ACETYLTHIO-17β-HYDROXY-4-ANDROSTEN-17α-YL)-PROPIONIC ACID γ-LACTONE]

FIG. 6 Structural formulae of aldosterone and its antagonists.

potassium by the kidney, its excretion is, in turn, increased by salt deprivation (85), excess potassium intake (86), or reduction in extracellular fluid (87).

The physiological importance of aldosterone has made it attractive to search for aldosterone antagonists as potential diuretic agents. The two approaches to this problem have been (*a*) to inhibit the elaboration or secretion of aldosterone by the adrenal cortex and (*b*) to inhibit or

If a salt of potassium or if acetazolamide is given to induce kaliuresis, the triazine depresses potassium excretion. Chlorazinil antagonizes the actions of salt-retaining adrenal steroids on electrolyte excretion and the glycogenic response to either 9α-fluorohydrocortisone or hydrocortisone (73). Szabo *et al.* (65) found the compound to increase the excretion of salt and water, but in a manner that suggested the two attributes to be independent of each other. There was no effect on glomerular filtration rate or on renal plasma flow in his experiments.

The diuretic action in man of both chlorazinil (74, 75) and amanozine (76), a closely related compound, has been studied by Ford and his associates, recently. They have indicated that the order of activity of chlorazinil at an effective dose of 600 mg. orally per day to man was essentially the same as for amisometradine (see previous section on aminouracils) and about half as active as meralluride, an organomercurial diuretic agent Whereas the minimal effective oral dose (150 mg., orally) of amanozine was half that for chlorazinil, the dosage response curve for the former agent was sufficiently flat that 300 mg. represented the maximally effective dose and 600 mg. caused severe gastrointestinal disturbances in nearly every case. He indicated that the saluretic action of amanozine was about one-third that of meralluride, but that 300 mg produced a greater water diuresis than either 600 mg. of chlorazinil, orally, or 2 ml. of meralluride given intravenously Hirshleifer (77) reported that in a series of edematous patients amanozine was an effective replacement for meralluride in about 31 6 per cent of cases and that it permitted a partial reduction in mercurial requirements in another 36 8 per cent of his group. In the aforementioned reports (74 to 76), Ford *et al.* pointed out that while neither of these two triazines influenced the serum electrolyte pattern deleteriously, both compounds caused an elevation of blood urea nitrogen without an increase in hematocrit, indicating nitrogen retention secondary to renal toxicity. This effect was reversible by withdrawing either compound. Both Miller & Ford (78) and Hirshleifer (77) have reported that a combination of amanozine and a carbonic anhydrase inhibitor, sulocarbilate (*p*-sulfamyl-2-hydroxyethylcarbanilate), was a more effective diuretic product in man than when either was administered alone

Thus, to date the triazines best studied in man influence water excretion more than salt, their order of activity is moderate, they tend to induce gastrointestinal complaints when the dosage is exaggerated, and the induced nitrogen retention portends a more generalized renotropic effect

ALDOSTERONE ANTAGONISTS

The demonstration of a salt-retaining factor in the urine of edematous patients by Deming & Luetscher (79), the isolation and characterization of aldosterone (Fig. 6) from adrenal venous blood by Simpson & Tait (80) in collaboration with Wettstein *et al.* (81), and its isolation from the urine of patients with nephrosis (25) or congestive heart failure (82) by

& Bethune (95) demonstrated in man the ability of the spirolactones (SC-8109) to antagonize the effect of a venoclysis of aldosterone on sodium excretion, and Chobanian *et al.* (96) reported that the same compound induced a marked sodium diuresis in a patient with Conn's syndrome (primary hyperaldosteronism). There has been a rapid clinical confirmation of the (variable) increase in sodium excretion, potassium retention or no effect, and a modest diuresis when these compounds or the more potent SC-9420 were administered especially to patients with cirrhosis, accompanied by ascites (97 to 100).

Both Conn *et al* (101) and Bolte and his associates (102) have reported that SC-8109 causes a decreased excretion of 17-hydroxycorticosterones in the urine, but Gantt & Dyniewicz (103) reported SC-9420 to have no effect on the excretion of these steroids Wiggins *et al* (104) noted no effect of SC-8109 on aldosterone excretion, but Bolte *et al.* (102) reported it to induce a significant increase in urinary aldosterone in two of three patients, with a marked rebound in one patient when the experiment was terminated. SC-8109 was reported by Wiggins *et al.* (105) to cause no change in glomerular filtration rate or renal plasma flow. Side effects noted have included lethargy, ataxia, and some gastrointestinal disturbances, although these have appeared infrequently.

As indicated by Liddle (7), these compounds have been effective only in those clinical edematous states where an increased excretion of aldosterone has been documented. Even so, this need not imply a specific aldosterone antagonistic action at the renal level. These compounds block the effects of mineralocorticoids on the tubular transport of electrolytes more generally. Kagawa *et al* (106) showed that SC-5233 was capable of blocking the renal effects of deoxycorticosterone, aldosterone, hydrocortisone, 21-deoxy-9α-fluorocortisone and 19-nor-deoxycortisone in rats.

The response to spirolactone therapy has been modest usually, and the maximal response may be delayed for hours or several days This relatively low order of activity is unfortunate, for the compounds to date represent a very difficult type of steroid chemistry However, their use in combined therapy with chlorothiazide may be practical. It has been well documented that the spirolactones act synergistically with chlorothiazide to give a greater saluresis and water output with a lesser effect on potassium output than can be obtained with a given submaximal dose of the latter agent (7, 97, 102, 103).

CONVENTIONAL CARBONIC ANHYDRASE INHIBITORS

The renal tubules, like the erythrocytes, gastric mucosa, brain, and the [illegible]

erythrocytes and its rapid exchange in the parenchyma of the lung.

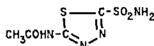
The importance of this enzyme to renal physiology was not appreciated

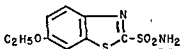
interferes with 11-, 17-, and 21-hydroxylations. Thorn *et al.* (89), Hertz *et al.* (90), and Gallagher and his associates (91) have demonstrated the inhibition of aldosterone excretion in man by amphenone. This suppression of aldosterone excretion is usually attended by an increase in the urinary elimination of sodium, but this is not invariably the case Thorn *et al* (92) cite three cases of "secondary" hyperaldosteronism, as evidenced by anasarca and increased excretion of aldosterone, in which amphenone depressed aldosterone excretion to normal but with an attendant significant natriuresis in only one instance. Lieberman (6) illustrated this same point (depressed excretion of aldosterone without a real increase in sodium excretion by amphenone) with a case presentation in his review on aldosterone. Actually, amphenone also suppresses the excretion of 17-hydroxycorticosterone and produces such generalized manifestations of toxicity (90) as to preclude its use except for experimental purposes.

In 1957, Kagawa, Cella & Van Arman (93) reported that two steroid spirolactones, SC-5233 and its 19-nor analogue (SC-8109) (see Fig 6), antagonized the effect of aldosterone or deoxycorticosterone acetate (DOCA) on the excretion of sodium and potassium in the adrenalectomized rat. This antagonism between the spirolactones and the salt-retaining hormones, wherein the inhibitor caused an increase in sodium (chloride) excretion and a decrease in potassium elimination, could be demonstrated to obtain at a relatively constant dosage ratio when the amount of DOCA administered to the rats was varied. The blocking action of the spirolactone could be overcome by the administration of excessive dosages of DOCA These data, and the supportive evidence that neither spirolactone influenced sodium or potassium excretion by the normal rat, led them to the proposal that the steroids acted as antagonists or blocking agents for aldosterone at the renal tubule in accordance with the principle of mass action Liddle (94) extended the observations of Kagawa *et al* (93) to the adrenalectomized dog, wherein either spirolactone caused an increase in sodium excretion and a retention of potassium when the animals were maintained on DOCA. When either compound was administered to patients in whom the excretion of aldosterone (or DOCA) might be thought to be elevated (as in congestive heart failure or nephrosis, in Addison's disease maintained on a high sodium diet plus DOCA, or in normal volunteers who received a low salt diet), they induced an increased excretion of sodium and a retention or no effect on potassium elimination. When administered to the Addisonian patient maintained only on a high salt intake, or when given to normal patients on a high salt diet, the compounds produced no effect on the excretion of sodium, presumably because of the absence or depression of aldosterone elaboration These experiments also served to indicate that the compounds inhibited the salt-retaining potassium-excreting action of aldosterone or DOCA on the renal tubules.

Within a few months, the basic observations of Kagawa *et al.* (93) and of Liddle (94) were amply substantiated. Taken out of sequence, Ross

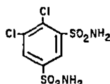
Although acetazolamide is well absorbed when administered orally and is safe and, although it has been employed in a variety of conditions of fluid retention from that associated with pregnancy (121, 122) to congestive heart failure (123 to 125), its utility has been limited. The limitations are referable to the pharmacological attributes previously mentioned. Thus, Counihan *et al.* (126) and Leaf *et al* (127) have pointed out that the moderate diuretic action of the compound in edematous patients may be attributed to the sharp limitation of reduction in extracellular fluid that can be induced by an increased excretion of bicarbonate without chloruresis A





ACETAZOLAMIDE
(2-ACETYLAMINO-5-SULFAMYL-1,3,4-THIADIAZOLE)

ETHOXZOLAMIDE
(6-ETHOXYBENZOTHIAZOLE-2-SULFONAMIDE)



DICHLORPHENAMIDE
(1,3-DISULFAMYL-4,5-DICHLOROBENZENE)

FIG 7 Structural formulae of carbonic anhydrase inhibiting diuretic agents

slight reduction in plasma CO_2 secondary to the action of acetazolamide renders the renal mechanism reversibly refractory to its influence and necessitates either an intermittent dosage schedule or the employment of submaximal dosage in order to obtain a sustained effect Finally, the general distribution of the compound and its effect on carbonic anhydrase other than in the kidney probably accounts for the paresthesias and drowsiness that it can induce, but which are reversible upon withdrawal of the drug The occurrence of renal calculi (128 to 130) and bone marrow depression (131 to 133) has been reported, but they are infrequent complications

At least two other compounds that are available in this category should be mentioned. Ethoxzolamide[a] (Fig. 7) appears to be about twice as potent as acetazolamide and to have both the same attributes and limitations of

[a] The trade name of the Upjohn Company for ethoxzolamide, 6-ethoxybenzothiazole-2-sulfonamide, is Cardrase.

until after the advent of sulfonamide chemotherapy. Early in the days of sulfanilamide administration, Southworth (108) and Strauss (109) noted that it induced acidosis secondary to an increased excretion of bicarbonate Several years later Mann & Keilin (110) pointed out that sulfonamides that contained an unsubstituted sulfamyl group in their molecule were capable of inhibiting carbonic anhydrase, but that if there was one or more substitutions on the sulfamyl-N group such a compound was inactive In turn, Hober (111) noted that some sulfonamides were capable of inhibiting the acidification of urine by the frog kidney. Pitts & Alexander (16) employed these basic observations to document their important concept regarding the ion exchange theory of urine acidification. It was their thesis that the hydration of carbon dioxide in the presence of carbonic anhydrase in the renal tubule contributed somehow to the availability of hydrogen ions, as by the dissociation of carbonic acid, for exchange with sodium contained in the glomerular ultrafiltrate This exchange of hydrogen for, primarily, sodium across the luminal border of the tubular epithelium was the critical factor in the ion exchange theory of urine acidification. They demonstrated that sulfanilamide decreased the acidity of urine and increased the excretion of sodium and bicarbonate ions, which they attributed to the inhibition of the carbonic anhydrase-dependent hydrogen ion exchange mechanism. There remained then the clinical demonstration by Schwartz (112) of the natriuretic-diuretic effect of sulfanilamide in patients having congestive heart failure, but the compound was too toxic to be employed generally for this purpose Recently, Berliner & Orloff (113) have reviewed comprehensively the basic aspects of the actions of carbonic anhydrase inhibitors.

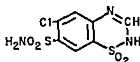
The search for a potent, relatively safe carbonic anhydrase-inhibiting diuretic agent reached its initial objective with the availability of acetazolamide[5] (Fig 7) The pharmacology of this compound remains today as first represented by Maren and his associates (114, 115). They showed that this agent was a potent carbonic anhydrase inhibitor, *in vitro*. When administered to dogs it caused an increase in the excretion of sodium and bicarbonate ions primarily, there being a lesser effect on potassium elimination, although this too was notable. It caused essentially no chloruresis. The natriuretic and the urinary alkalizing effects were more prominent under conditions of alkalosis and were negated by acidosis. The compound induced a metabolic acidosis, secondary to the loss of sodium bicarbonate Under such conditions of acidosis the compound became ineffective, the reasons for which Gilman (116) has discussed in his review of the mechanism of action of carbonic anhydrase inhibitors.

Acetazolamide is widely distributed in the body in sufficient concentration to inhibit carbonic anhydrase in several tissues. Thus, it is employed in the management of glaucoma (117, 118) and, to a lesser extent, in the treatment of certain types of epilepsy (119, 120).

[5] The trade name of *Lederle Laboratories Division, American Cyanamid* Co for acetazolamide, 2-acetylamino-1,3,4-thiadiazole-5-sulfonamide, is Diamox.

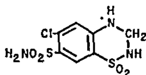
cology will serve as the prototype for discussion of these several compounds. The following characteristics of the compound were summarized by Beyer (154).

The preponderant renotropic characteristic of chlorothiazide is the pro- [illegible] the urine becomes alkaline Thus, the compound behaves in the manner of the organomercurial diuretic agents with respect to saluresis at low dos-



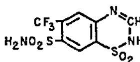
CHLOROTHIAZIDE

(6-CHLORO-7-SULFAMYL-1, 2,4-BENZOTHIADIAZINE-1,1-DIOXIDE)



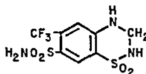
HYDROCHLOROTHIAZIDE

(6-CHLORO-7-SULFAMYL-3, 4-DIHYDRO-1,2,4-BENZO-THIADIAZINE-1,1-DIOXIDE)



FLUMETHIAZIDE

(6-TRIFLUOROMETHYL-7-SULFAMYL-1,2,4-BENZO-THIADIAZINE-1,1-DIOXIDE)



HYDROFLUMETHIAZIDE

(6-TRIFLUOROMETHYL-7-SULFAMYL-3,4-DIHYDRO-1, 2,4-BENZOTHIADIAZINE-1,1-DIOXIDE)

Fig 8 Structural formulae of chlorothiazide and related compounds

ages, the more characteristic action of carbonic anhydrase inhibitors becoming evident as the dosage is increased. This important distinction of predominant chloride elimination for chlorothiazide versus the predominant bicarbonate excretion for acetazolamide and similar agents undoubtedly accounts for its greater utility, the relative lack of refractoriness and a seeming uncertainty with regard to its mode of action

Another characteristic of chlorothiazide which serves to distinguish it from acetazolamide and the organomercurial agents is the persistence of its action under conditions of ammonium chloride acidosis or sodium bicarbonate alkalosis in the dog It will be recalled that acetazolamide is essentially inactive under conditions of acidosis (155), being most active

acetazolamide with respect to its diuretic action (134 to 138) and in the

order of activity as a carbonic anhydrase inhibitor *in vitro* and the distribution characteristics of acetazolamide in the body. Thus, its utility in glaucoma (140 to 143), as a diuretic agent (144), and in the management of respiratory acidosis (145) is similar to that for acetazolamide, except that dichlorphenamide is considerably more potent on a dosage basis.

Dichlorphenamide represents a transitional agent between the renal characteristics of acetazolamide and of chlorothiazide. It causes the excretion of both bicarbonate and, to a lesser extent, chloride to attend the natriuretic effect. Thus, in the dog it is difficult to induce refractoriness to the agent, under conditions that render acetazolamide essentially inactive (146).

CHLOROTHIAZIDE AND ITS CONGENERS

A study of the chemical structure of the compounds represented in Figure 8 reveals that chlorothiazide[8] and its several congeners that are available currently share a sulfamyl group in common with those agents, such as acetazolamide, illustrated in Figure 7 as conventional carbonic anhydrase inhibitors Toward the end of the section on Carbonic Anhydrase Inhibitors, it was mentioned that dichlorphenamide possessed the same order of carbonic anhydrase inhibitory action as did acetazolamide and yet was capable of producing an increase in chloride excretion along with a preponderant elimination of bicarbonate as the anion that attended the excretion of sodium and potassium. Still earlier, Beyer (147) pointed out that a very simple analogue of sulfanilamide, *p*-carboxybenzene sulfonamide (CBS), was capable of causing a chloruresis to accompany its natriuretic effect. This action of the compound was substantiated clinically by Merrill (148) and by Lindsay & Brown (149). However, this compound was poorly absorbed when administered orally to man and had an intrinsic low order of activity, as compared with the more recent saluretic agents of the chlorothiazide type.

A continuation of the research stemming from the structure-activity relationships noted for CBS led Novello & Sprague (150) to synthesize a series of benzothiadiazines, from which chlorothiazide was selected by Beyer, Baer, and their associates (151 to 153) for clinical trial. Since the more recent analogues presently available to the physician differ essentially only quantitatively with respect to the action of chlorothiazide, its pharma-

[7] The trade name of Merck Sharp & Dohme, Division of Merck & Co, Inc for dichlorphenamide, *1,3-disulfamyl-4,5-dichlorobenzene, is Daranide*

[8] The trade name of Merck Sharp & Dohme, Division of Merck & Co, Inc. for chlorothiazide, 6-chloro-7-sulfamyl-1,2,4-benzothiadiazine-1,1-dioxide, is Diuril in the United States and Canada, Saluric in the United Kingdom, Chlotride in Europe, the Near East, and the Far East, and Clotride in Latin America and Portugal

the presently available agents and those known to be on clinical trial with respect to their kaliuretic effect. Except for the weakness that may attend a depletion of potassium, the consequences of this effect have not been [illegible]

especially enthusiastic overdosage, these compounds can induce a hypochloremic alkalosis and, more rarely, a hyponatremia (168). These results are, in effect, testimony to the potency of these agents. The hypokalemia can be negated by the employment of low dosages of the compounds wherein such is sufficient, by the recommendation of fruit juice, or by the administration of potassium chloride. Neither of these latter two measures may be sufficient to maintain a normal potassium blood level during the aggressive therapy of cirrhosis or nephrosis. Occasionally, in the treatment of cirrhosis, the hypokalemia may be attended by an increase in ammonium blood level (169). The cause for this is not well understood, although characteristically an increase in the excretion of sodium and potassium is attended by a reciprocal decrease in the amount of ammonium ion excreted by the kidney. Hypersensitivity to chlorothiazide has been rare, but has been reported. Withdrawal of the compound results in a satisfactory abatement of signs and symptoms (170 to 173).

The mode of action of chlorothiazide and its congeners is not thoroughly understood. All of these compounds are active carbonic anhydrase inhibitors. Chlorothiazide is about one-tenth as potent a carbonic anhydrase inhibitor *in vitro* as acetazolamide and, in turn, hydrochlorothiazide is approximately one-tenth as potent as chlorothiazide. The trifluoromethyl agents, in general, are weaker inhibitors of carbonic anhydrase than are their chloro analogues (174). The fact that hydrochlorothiazide is a more potent saluretic agent *in vivo*, and a less active carbonic anhydrase inhibitor *in vitro*, than chlorothiazide has lent support to the supposition that the saluretic action of these agents may be dissociated from their ability to inhibit that enzyme On the other hand, all active saluretic agents of the benzothiadiazine type either have the free (unsubstituted) sulfamyl group (175) or carry substitutions that can be hydrolyzed in the body to yield the free or carbonic anhydrase inhibitory sulfamyl group (176).

It seems certain, as represented by Beyer (154), by Pitts *et al.* (177), and by Ford & Rochelle (178), that the mode of action of chlorothiazide differs from that of the organomercurial agents, although both apparently inhibit mechanisms that contribute to the energy requirements or availability of hydrogen ion for exchange with sodium in the nephron It is generally believed that chlorothiazide shares with acetazolamide the ability to inhibit the carbonic anhydrase component of the ion exchange mechanism of hydrogen for sodium across the tubule. Because of the fact that the

under conditions of bicarbonate alkalosis. Conversely, the mercurial diuretic agents are potentiated by ammonium chloride and are rendered, for the most part, inactive under conditions of alkalosis (156). The fact that chlorothiazide increases the excretion of both sodium and, to a lesser extent, potassium distinguishes it from the aldosterone antagonists which induce a natriuresis without evoking a kaliuresis (93, 94). Indeed, the aldosterone antagonists characteristically may induce a retention of potassium. On the other hand, both types of agents cause an increase in chloride elimination.

Chlorothiazide and its congeners are well absorbed when administered orally. Indeed, they are essentially as active when administered orally as when injected intravenously. In general, the distribution of these compounds is limited inherently to extracellular fluid (157), there being some differences in their absorption on plasma protein. The importance of this extracellular distribution is reflected in the relative lack of side effects such as obtain for those carbonic anhydrase inhibitors that are distributed into the brain, erythrocytes, and other tissues. This factor of limited distribution is responsible for the fact that these compounds have little or no pharmacodynamic effects other than as represented by electrolyte transport.

Although the metabolism of the trifluoromethyl analogues of chlorothiazide and hydrochlorothiazide[9] is not well understood, the chloro progenitors are rapidly excreted as such when administered intravenously according to Baer *et al.* (157, 158). In bilaterally nephrectomized dogs, both agents are excreted to some extent at least by way of the hepatic system, but the predominant elimination of these compounds is by glomerular filtration and renal tubular secretion. The renal tubular elimination appears to be by the same mechanism as for *p*-aminohippurate with which they compete for secretion (159). The tubular secretion of these compounds can be inhibited by probenecid.[10]

The acute toxicity of all of these compounds is so low as to be unimportant except as a safeguard to the consequence of their inadvertent or deliberate overdosage (160). Thus, it has been possible to administer as much as 1000 mg/kg. of chlorothiazide or hydrochlorothiazide to dogs for a period of months. Dogs tolerate as much as 500 mg/kg/day of chlorothiazide intravenously over a period of weeks (161). The important untoward effects of these several compounds is inherent in an exaggeration of their effect on electrolyte balance. The more notable of these effects is the hypokalemia which any of these agents can induce secondary to a kaliuresis (162 to 167). There seems to be no important difference between

[9] The trade name of Merck Sharp & Dohme, Division of Merck & Co, Inc. [illegible] 6-chloro-7-sulfamyl-3,4-dihydro-1,2,4-benzothiadiazine-1,1- [illegible] Ciba Pharmaceutical Products Inc. for

[10] The trade name of Merck Sharp & Dohme, Division of Merck & Co, Inc for probenecid, *p*-(di-n-propyl-sulfamyl)-benzoic acid, is Benemid.

to include almost all types of fluid retention of systemic or local nature that could be construed to be secondary to decreased excretion of salt These conditions have included the edema accompanying cardiac decompensation (192 to 195), nephrosis (192, 196), and cirrhosis (192, 197, 198). Fluid retention accompanying premenstrual tension or pregnancy has responded well to therapy with these agents (199 to 203), as have certain types of local edema (204) and angina pectoris (205) More recently, chlorothiazide has been reported to influence beneficially certain cases of acne (206, 207).

Hydrochlorothiazide (see Fig. 8) has been introduced recently as having at least a tenfold greater order of activity, as represented by dosage, when compared with chlorothiazide. There is reasonably good agreement between Baer *et al.* (158), De Stevens and his associates (208), and Barrett *et al* (190) that in dogs hydrochlorothiazide is between 6 and 10 times as active as chlorothiazide. In rats, Renzi *et al.* (209) and Goldberg & Hwang (210) have shown a greater variance in the order of potency (up to twentyfold) of hydrochlorothiazide, as compared to chlorothiazide. There seems to be a somewhat greater chloruretic-to-natriuretic effect for hydrochlorothiazide, as noted by Baer *et al* (158) and Barrett *et al.* (190) in dogs, and by Ford (211) in man. Interestingly, as the dose response for hydrochlorothiazide or hydroflumethiazide[11] is followed to excessive dosage, there is a secondary reduction in the reabsorption of sodium and chloride that is not reflected in the glomerular filtration rate. This was noted by Beyer & Baer (174) to obtain in dogs and by Moyer *et al.* (212) in man

The clinical utility of hydrochlorothiazide appears first to have been reported by Fuchs *et al* (213) and by Herrmann and his associates (214). In general, its use in congestive heart failure, in cirrhosis, and in the management of hypertension seems to be essentially the same as for chlorothiazide at approximately one-tenth the dosage of that compound (215, 216) The hypokalemia and the occurrence of hypochloremic alkalosis also have been noted for this agent, as indicated in the above references.

Flumethiazide[12] and hydroflumethiazide have been reported by Beyer & Baer (174), by Ford (217), and by Tisch *et al* (218) to be slightly less active than their corresponding chloro analogues Flumethiazide is slightly less active than chlorothiazide, and hydroflumethiazide is less active than hydrochlorothiazide, although Kobinger & Lund (219) have shown hydroflumethiazide to be more active than chlorothiazide in rats.

Although the clinical utility of the trifluoromethyl congeners of chlorothiazide remains to be reported in any detail, such pharmacology of the

[11] The trade name of Leo Pharmaceutical Products for hydroflumethiazide, 6-trifluoromethyl-7-sulfamyl-3,4-dihydro-1,2,4-benzothiadiazine-1,1-dioxide, is Rontyl.

[12] The trade name of E R. Squibb & Sons, Division of Olin Mathieson Chemical Corp, for flumethiazide, 6-trifluoromethyl-7-sulfamyl-1,2,4-benzothiadiazine-1,1-dioxide, is Ademil

tubular secretion of chlorothiazide appeared to be the same as that for *p*-aminohippurate, Beyer (154) proposed that the site of action of chlorothiazide was, for the most part, in the proximal convoluted tubule, and this has been substantiated by the stop-flow technique employed by Vander and his associates (179) and by Kessler *et al.* (220).

Laragh (180) and Blackmore (181) have distinguished between the strictly saluretic action of chlorothiazide and the fact that the organomercurial agents produce an increase in free water clearance attending their saluretic action In other words, the diuresis induced by chlorothiazide and its analogues seems to be on an osmotic basis equivalent to their saluretic effect, whereas the mercurials are capable of increasing water output over and above the elimination of salt. If one considers, as represented in the introductory remarks, that under conditions of fluid retention there may be a still greater retention of salt than water, and that the accumulation of fluid is more frequently secondary to the decrease in excretion of the electrolytes, the benzothiadiazines might be hypothesized to be the more physiologic diuretic agents

The antihypertensive action of these compounds (182 to 185) appears to be secondary to their effect on electrolyte balance This action has been interpreted variously by several investigators Beavers & Blackmore (186) and Preziosi *et al* (187) reported that large doses of chlorothiazide administered intravenously to dogs caused a decrease in response to the intravenous administration of epinephrine and norepinephrine. Likewise, Merrill *et al.* (188) reported that both chlorothiazide and a low salt diet decreased the responsiveness of patients to both the norepinephrine pressor response in the presence of ganglionic blocking agents and to tilting Interestingly, Beavers (189) observed that in the nephrectomized dog chlorothiazide was capable of decreasing the pressor response of epinephrine or norepinephrine Barrett *et al* (190) have extended this general type of observation to show that when hydrochlorothiazide was administered intravenously in substantial amounts it increased slightly the depressor response to hydralazine and to histamine Although there is some evidence to indicate that chlorothiazide influences the distribution of sodium and potassium on the two sides of the cell membrane of, particularly, the arterial musculature, Dustan *et al* (191) are of the opinion that the antihypertensive effect of chlorothiazide is the result of the reduction in circulating blood volume that attends its diuretic effect They consider that under the circumstances of the saluretic-induced oligemia there is an intensification of vasomotor tone and that the response of antihypertensive agents that influence adrenergic motivation of vasomotion is facilitated Regardless of interpretation, it seems well established that salt restriction or saluresis is capable of lowering blood pressure and of exaggerating the hypotensive response to most types of antihypertensive therapy, including ganglionic blocking agents, reserpine, hydralazine, and the monoamine oxidase inhibitors

The clinical utility of chlorothiazide and its congeners has been extended

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agents as is known indicates that they should have much the same type of utility and effect of salt and potassium excretion as obtain for chlorothiazide and hydrochlorothiazide. Thus, one might expect the incidence of hypokalemia and of other electrolyte disturbances to be essentially the same for these agents as for chlorothiazide and hydrochlorothiazide

In summary, chlorothiazide has been by far the most intensely employed of the benzothiadiazines, and its utility and safety are well documented Hydrochlorothiazide, from a dosage standpoint, is some 10 times as potent as chlorothiazide. However, the two compounds are equally effective when employed in equivalent dosages. Both of these compounds cause a profound saluresis of much the same duration when administered orally Both agents cause a hypokalemia secondary to an increase in excretion of potassium in some patients, especially when employed at high dosages The trifluoromethyl analogues of these two agents are slightly less active by critical assay than are their chloro analogues· They seem to offer no advantage over chlorothiazide or hydrochlorothiazide from the standpoint of utility or safety.

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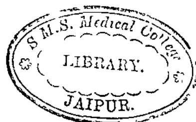
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